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REDAKTÖR: ARNE MÜNTZING

BAND 44: 1 1958

BERLINGSKA BOKTRYCKERIET, LUND

HEREDITAS

a periodical devoted to the publication of original research in genetics and cytology, is published by the MENDELIAN SOCIETY of Lund, Sweden. All contributions are written in English, German or French, illustrated when necessary, with adequate text figures and plates. The current volume (44) will contain about 500 pages, issued in four numbers. Price *Forty (40) Swedish Kronor per volume.* Postage free.

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(Vols. 13—18, 20—26, 33—36, 38 and Suppl. Vol. 1949 are entirely out of print.)

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A STUDY IN HYACINTHUS ON CHROMOSOME SIZE AND BREAKABILITY BY X-RAYS

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I. INTRODUCTION

IT is known that there are big differences between species as regards their sensitivity towards X-rays and other ionizing radiations. The causes of these differences are to a large extent unknown. There are, however, a few factors whose importance in this connection may be more obvious. One factor of this kind is *polyploidy* and another factor which seems to be of some importance is *chromosome size.*

Polyploids usually show less phenotypical damage than diploids, when given the same X-ray dose, a fact which certainly is due to each gene locus being repeated several times in the polyploids (MÜNTZING, 1941, 1942; SMITH, 1946). Any damage induced at a certain locus or chromosome region will, for this reason, be more efficiently counterbalanced in polyploids than in diploids by the presence of other homologous loci or chromosome regions.

Studies on the frequency of radiation-induced chromosome structural changes per cell in diploids and in polyploids demonstrate that these changes increase more or less proportionately to the degree of polyploidy (*e. g.* FRÖIER, GELIN and GUSTAFSSON, 1941; FRÖIER, GUSTAFSSON and TEDIN, 1942; SMITH, 1946; CONGER and JOHNSTON, 1956). This is obviously due to the fact that the chromosome material is about equally sensitive in the diploids and in the polyploids, but with a higher quantity of this material present per cell in the polyploids.

MARSHAK (1937) made some observations suggesting that the frequency of X-ray-induced chromosome disturbances per cell increases with an increased chromosome size. His study was undertaken on a heterogeneous group of materials, *viz. Lycopersicum esculentum, Mus musculus, Pisum sativum, Allium cepa* and *Vicia faba.* He investigated

the anaphases in fixations made 3 hours after the irradiation. It is obvious that the disturbances to be found at such a short time after the treatment are to a certain extent of the kind that is usually called the »primary effect» of irradiation. The anaphase disturbances, in *Allium* at least, appear at this fixation time to consist of a mixture of pseudochiasmata and chromatid (including iso-chromatid) changes with some stickiness (KIHLMAN, 1955). In any case, as the pseudochiasmata most probably represent a kind of real chromosome breakage effect (LA COUR and RUTISHAUSER, 1953; ÖSTERGREN and WAKONIG, 1954; KIHLMAN, 1955), it seems reasonable to regard the disturbances studied by MARSHAK (1937) as due mainly to chromosome breakage. MARSHAK correlated the frequency of the disturbances with chromosome size measured as the length of the chromonemata inside the metaphase chromosomes. The estimation of the actual length of the chromonema itself at this stage must be very difficult, and the technique of revealing the spirals in these somatic metaphase chromosomes does not appear very satisfactory. He interpreted the increased effect that accompanied the increased chromosome size as due to the larger radiation target per cell.

According to certain recent studies the size of the chromosomes seems to be an important factor influencing the radiosensitivity of plant species (SPARROW and CHRISTENSEN, 1953; NYBOM, 1956). These investigators studied the growth inhibition and sterility induced by treatment with chronic gamma radiation from Co^{60}. Species having big chromosomes appeared to be much more sensitive than those having small ones. This difference in radiation damage is in good agreement with the interpretation that the growth inhibitions in such experiments are, to a large extent, caused by chromosome breakage, an opinion expressed by NYBOM (1956).

A difference in radiosensitivity between species of different chromosome size might be expected from the target interpretation of the origin of chromosome breaks in experiments with ionizing radiations. According to this theory a chromosome or chromatid break is caused by the passage of an ionizing particle through the chromosome or chromatid (LEA and CATCHESIDE, 1942; LEA, 1946; CATCHESIDE, 1948; KAUFMANN, 1954; GILES, 1954). In the case of X-rays only the end of the electron track has a sufficient density of ionizations to cause a break, but more densely ionizing particles, such as the protons of experiments with neutron irradiations, may break a chromosome whenever they pass through it. At least 15—20 ionizations of such an ionization track should occur within a chromatid in order to break it, as calculated for *Tradescantia* (LEA

and CATCHESIDE, 1942; LEA, 1946). It would be interesting to know whether a lower number of ionizations would suffice to break chromosomes that are narrower than those of *Tradescantia*. In any case, it seems clear that the chance of hitting a chromosome by an ionization track should be less for small chromosomes than for large ones. For this reason we should expect fewer chromosome breaks per cell in species with small chromosomes than in those with larger chromosomes.

As far as we can see, it is possible that chromosome size could be a factor of importance also in cases where the breaks are induced by an indirect mechanism. In such a case a break may be initiated when a radical or a reactive molecule enters into reaction with the chromosome material at some point. Probably the break then results from a kind of chain reaction across the chromosome, the chain starting from the point where the chromosome was attacked by the radical. If the concentration of the radical is the same, the chance that a reaction will take place between it and the chromosome material will increase in direct proportion to the amount of chromosome material that is present.

It is the purpose of the present paper to contribute observations on the frequency of X-ray-induced chromosome fragmentation in two species of *Hyacinthus* which differed in chromosome size. It was found that much more fragmentation per cell was obtained in the species with the larger chromosomes than in the species with the smaller ones, a result in agreement with the considerations presented above. Naturally it is not permissible to generalize from an investigation of this kind on a single species pair. We realize that it will be necessary to confirm the present result on a whole series of species pairs before its general validity can be accepted. We believe that the best test of the effect of chromosome size in the respect studied is to make comparisons between members of pairs of related species, each pair consisting of one species with smaller chromosomes and one with larger ones.

II. THE CYTOLOGY OF OUR MATERIALS AND THE PROBLEM OF DIFFERENCES IN CHROMOSOME SIZE

The materials used in our study were *Hyacinthus dalmaticus* (*Bellevalia dalmatica*) and *Hyacinthus orientalis*. The bulbs of the first species were obtained from Messrs. C. G. van Tubergen, Ltd., Zwanenburg Nurseries, Haarlem, Holland. In the case of *Hyacinthus orientalis* we used the diploid variety »White Roman Hyacinth». Bulbs of Dutch

origin were bought from the seed company Normanns Fröhandel, Lund, Sweden.

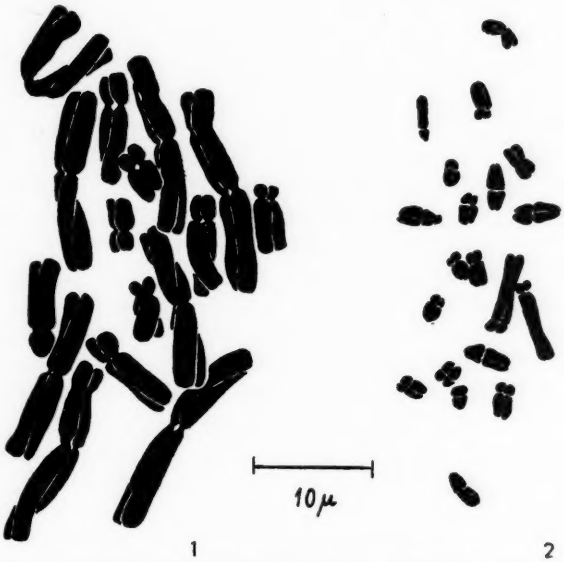
In our investigations we used root tip mitoses from roots formed on the bulbs when these were placed on tap water. *Hyacinthus orientalis* has $2n=16$ chromosomes which are well-known from numerous studies by different authors. They are very big (Fig. 1) and belong to three different size groups, 4 long, 2 medium-sized and 2 short pairs. *Hyacinthus dalmaticus* has not been investigated cytologically before. According to our study it has $2n=20$ chromosomes which are much smaller than in the other species (Fig. 2). There is a considerable variation in length between the different pairs.

Figures 1 and 2 are drawn from aceto-orcein squashes of roots in which the chromosomes have been supercontracted by means of special treatments. The treatments were applied in order to get mitotic figures in which the chromosomes showed a better separation from one another and where their morphological features were more distinct. The differences in size are as clear as in mitoses that are untreated.

The roots of *H. dalmaticus* had been treated for 3 hours with a solution of 8-oxy-quinoline containing 0.002 mols per litre. The oxy-quinoline pre-treatment method was introduced by TJIO and LEVAN (1950). The roots of *H. orientalis* had been treated with a 0.1 % solution of colchicine for 16 hours at room temperature (ca. 20° C), followed by treatment with a unimolar solution of acetamide for about 6—7 hours at 13° C. During this treatment the roots were still attached to their bulb. This combined treatment with colchicine and acetamide is a new method by G. ÖSTERGREN, which has not been published before. It often gives chromosomes well suited for a study of their morphological characteristics. The acetamide treatment should be performed at a relatively low temperature, however, and caution should be used not to apply it for too long a time, in order to avoid killing the roots and causing necrotic changes in the cells.

The two species were subjected to different methods of pre-treatment because they did not respond well to the same kind of treatment. Thus, colchicine treatment causes a clumping of the chromosomes of *H. dalmaticus* which makes them difficult to spread. With oxy-quinoline treatment of *H. orientalis* the number of divisions is low and the degree of supercontraction of the chromosomes is not strong enough to let them lie sufficiently free from one another to give many good cells.

The difference in chromosome size between the two species in Figs. 1—2 is not caused by the fact that different methods of pre-treat-



Figs. 1—2. The chromosomes of *Hyacinthus orientalis* (Fig. 1) and *H. dalmaticus* (Fig. 2). The chromosomes have been subjected to treatments causing them to supercontract in order to bring out the details more clearly (see text). Aceto-orcein squashes.

ment were used in the two cases. This difference is just as obvious also in the case of untreated chromosomes. But the possibility exists, of course, that the exact quantitative degree of the difference may be influenced by the different treatments. The chromosomes of *H. orientalis* are both longer and broader than those of *H. dalmaticus*. With the treatments used in Figs. 1—2 the chromosomes of *H. orientalis* have a volume that, roughly estimated, may be 10—11 times larger than that for the other species.

The existence of a similar difference in chromosome size is obvious at all stages of the mitotic cycle in which the chromosomes can be seen, although exact quantitative comparisons cannot be made at other stages than meta- and anaphase. When the root tips are prepared as Feulgen squashes, it can be seen that the amount of Feulgen-staining material (DNA) is much smaller in the resting nuclei of the small chromosome species than in those of the big chromosome species. The difference is so great that it can be easily ascertained by ordinary microscopic examination of the slides. No quantitative spectrophotometric measure-

ments are necessary for this purpose. This fact suggests that there is a big difference also in the amount of chromosome material present in the resting nuclei. This fact may be of importance, as the great majority of the cells in which we studied chromosome breakage must have been in the resting stage when they were X-rayed. The resting nuclei and also the cells are smaller in the small chromosome species. Although no quantitative study has been made of the nuclear size, it appears that the difference in this case is not as big as that in chromosome size. Some results concerning cell size are given below.

It is a well-known fact that, in some cases, there are big differences in chromosome size between related species. There are some different alternatives that may be suggested to explain this phenomenon. One possible interpretation would be that the big chromosomes might be enlarged by the presence of various inert materials. As heterochromatin in some cases, at least, appears to be of low genetic activity, differences in chromosome size might sometimes be connected with differences in the amount of heterochromatin. For this reason some observations were made on the amount and distribution of heterochromatin in the two species.

Hyacinthus orientalis is practically free from heterochromatin. There are only some very small heterochromatic regions in the immediate vicinity of the centromeres and, furthermore, the nucleolar chromosome has a small chromocentre in contact with the nucleolus. Otherwise, the nuclei have a homogeneous, euchromatic appearance. The prophase chromosomes of these root tip mitoses show a uniform thickness and staining throughout their length. *H. orientalis* appears to be an example of a species which does not show a heteropycnosis of the gradient type which LIMA-DE-FARIA has described *e. g.* for rye (1952).

The other species, *Hyacinthus dalmaticus*, shows many chromocentres in the resting nuclei, some of which are small, dense and distinct, whereas others are large, diffuse and indistinct. The prophases show regions which manifest a strongly positive heteropycnosis. These are probably located around the centromeres. These thick and strongly staining regions are connected to the thin and weakly staining chromosome ends by a »gradient» of chromosome regions showing all intermediates in these properties. It would appear that in this species we have a similar gradient in the staining properties and the thickness of the chromosome to that which is known for *Agapanthus* (DARLINGTON, 1933; LIMA-DE-FARIA, 1953) and *Secale* (LIMA-DE-FARIA, 1952). This gradient obviously represents a kind of heterochromatin differentiation.

Thus, a comparison shows that the small chromosome *Hyacinthus* species has more heterochromatin than the other species. The difference in chromosome size obviously cannot be due to an accumulation of more heterochromatin in the species with the large chromosomes. The possibility still remains that the chromosomes of the big chromosome species may contain some kind of more or less inert material which is not present in the other species. A property of interest, when we consider this question, is cell size, as will now be briefly discussed.

It is a well-known fact that, parallel with the increase in chromatin mass in a polyploid series, there is also an approximately proportionate increase in cell volume. Obviously this increase may be regarded as a self-regulation of the cell for the purpose of maintaining a proper balance between the physiological activities of the nucleus and the cytoplasm. If the chromosome size were increased by the presence of physiologically inert material, then we should expect this increase to take place without a corresponding increase in cell size. For this reason, it is of interest in the present connection that diploid species having bigger chromosomes than other diploids related to them have also an increased cell volume. This correlation was demonstrated by NAVASHIN (1931) for a series of diploid species of *Crepis*. Similar cases have been reported by others. Thus, SCHWANITZ and PIRSON (1955), in a study on many species, compared intraspecific races (or other closely related types) differing in their degree of *gigas* appearance. They found that in some species the giant types had an increase in size of chromosomes and of cell volumes (*e. g.* in *Allium porrum*) while in other cases the giant properties were due to other circumstances.

We have tested the difference in cell size between *H. dalmaticus* and *H. orientalis* by means of measurements of pollen grains and stomata of the two species. The stomata were measured on the upper leaf epidermis of corresponding leaves, one leaf from a single individual of each species. Only morphologically normal pollen grains were measured. The frequency of normal pollen was quite high in the samples used. The differences can be seen in Table 1. These differences were confirmed by the observation of big differences in size of the root tip cells studied in our X-ray experiments. In the roots, however, no measurements were made. The figures for relative volume in Table 1 have been calculated from those for relative length on the basis of the idea that the cell shape is the same in both species, a procedure that may be an acceptable approximation. Obviously it would be valuable to have measurements from many different individuals of each species, but we think that the

TABLE 1. *Comparisons between two Hyacinthus species with respect to the sizes of their pollen grains and stomata.*

	Pollen grains			Stomata		
	Length in μ	Relative length	Relative volume	Length in μ	Relative length	Relative volume
H. dalmaticus	30.40	1	1	32.31	1	1
H. orientalis	37.08	1.22	1.81	47.17	1.46	3.11
Significance test	Variance ratio = 172.7 $N_1 = 1$ $N_2 = 47$ $p < 0.001$			Variance ratio = 813.1 $N_1 = 1$ $N_2 = 52$ $p < 0.001$		

The measurements are based on a single plant of each species. For this reason they do not prove a difference of general validity between the species and should only be regarded as a preliminary study.

present results may suffice to give a preliminary idea of the difference between the two species. The observation that the difference in size is much smaller for the pollen grains than for the stomata is in good agreement with the observations by SCHWANITZ and PIRSON (1955) on various other materials.

Our observations on the difference in cell size support the interpretation that the increase in chromosome size cannot be due merely to an accumulation of inert material in the chromosomes of the big chromosome species. The difference in cell size appears, however, not to be as great as the difference in chromosome size. The reason for this *may* be that some of the additional chromosome material in the big chromosome species may be more or less inert, but it *may* also be due to other circumstances.

It has been mentioned that the increased cell size which seems to be conditioned by the larger chromosomes suggests that the additional chromosome material is not inert. It should be pointed out that the activity of this material is a *physiological* one, which *need not necessarily* imply also a genic activity. But it seems a reasonable assumption to suppose that this physiological activity might be the consequence of a genic activity in the additional chromosome material.

It does not seem probable that differences in chromosome size between related species are due to the presence of many more gene loci in the one species than in the other. When big differences in chromosome size are found between different varieties of the same species (as

in *Allium porrum*, SCHWANITZ and PIRSON, 1955) or even between different tissues of the same individual (as in *Rhoeo discolor*, GEITLER, 1940), a difference in the number of gene loci is, of course, excluded. It should be noted that in these cases (*Allium* and *Rhoeo)* there is not merely a difference in the degree of contraction of the chromosomes that might be attributed to differences in their spiralization, but also in chromosome volume, the larger chromosomes being thicker as well as longer. Similar differences in chromosome size between different tissues were also reported by CZEIKA (1956) for various species of the *Compositae* and by GEITLER (1955) who found that the mitotic chromosomes of the young endosperm in *Allium ursinum* were much larger than the ordinary mitotic chromosomes.

One possible interpretation of such differences in chromosome size is that they are due to a kind of polyteny similar to that known from the giant salivary gland chromosomes of the *Diptera.* The chromonema is probably composed of a bundle of elementary chromosome fibrils (called monids by DARLINGTON, 1950), an opinion held by many authors, *e. g.* RIS (1957). A variation in the number of these fibrils within the chromonema could be at least one of the causes for differences in chromosome size, a possibility discussed by GEITLER (1940, 1955), MECHELKE (1952), SCHWANITZ (1953), HUGHES-SCHRADER and SCHRADER (1956), RIS (1957) and others.

We would like to direct attention to the fact that if the fibril bundle which constitutes the chromonema is present in a spiralized condition, then an increase in the *number* of the elementary fibrils is capable of producing not only a thicker chromosome, but also a longer one, even though the length of the elementary fibrils may remain the same. This can be easily demonstrated in a simple experiment with strings (Fig. 3). This factor could work not only on the spiralized chromosomes of metaphase and anaphase, but it could also influence the length of the chromonema itself if this contains a submicroscopic helix or is composed of units that are spiralized in this way. Could it be that this is the cause of that gradual increase in length of polytenic resting stage chromosomes (»giant chromosomes»), which normally accompanies their growth in thickness? This increase in length is known from the *Diptera* and was also observed in higher plants by TSCHERMAK-WOESS (1957).

A chromosome which in this way represents a higher degree of polyteny (or polymery as some authors say in this connection) would obviously represent a larger target for ionizing radiations, very much in

Fig. 3. A model experiment with strings demonstrating that a growth in thickness of a chromonema (or subchromonema), *e. g.* produced by polyteny, can result in a chomonema (or subchromonema) that is not only *thicker* but also *longer*. This effect is obtained if there is a submicroscopic spiral structure in the chromonema (subchromonema). — Strings of the same length but differing in thickness have been coiled in a closely-packed helix around a thin wire.

the same way as a polyploid nucleus represents a larger target than a diploid nucleus. Thus, the number of breaks per nucleus would increase for practically the same reasons as in the case of polyploidy. The essential difference from polyploidy in the present connection would lie in the fact that the reduplicated chromosome material in a polyploid is located in separate chromosomes which respond individually towards breakage, while in polymeric chromosomes the reduplicated material is all located in a single chromosome, acting as a unit towards breakage. For this reason, the damage induced in a polyploid is buffered by the presence of undamaged reduplications of the same loci that were influenced. In polymeric chromosomes there will be no buffering of this kind, as the reduplicated material is all damaged in the same way.

It may be difficult to understand how it is possible for a polymeric chromosome to react as a single unit towards breakage. But, as a multiple structure is most probably present also in »normal» (or low-polymeric) chromosomes, an increase in the degree of multiplicity does not introduce a new problem. As pointed out by ÖSTERGREN and WAKONIG (1954) the common reaction of the chromatid (or early resting stage chromosome) as a single unit towards breakage may be due to a purposeful adaptation of this kind of structure to behave as a unit during chiasma formation at meiosis. Part-chromatid crossing-over would lead to serious difficulties at the anaphase separation.

It is quite possible that the multiplicity in structure of a chromosome which makes it appear at mitosis larger in size in one species than in another is, partially at least, of a different kind than that which con-

ditions the enormous growth of the polytenic salivary gland chromosomes. The reference made here to the possible relations between these phenomena should not be taken to mean that we assume that they are identical.

III. THE X-RAY EXPERIMENT

The bulbs used in the experiment were cultivated in a tank with running, aerated tap water maintained at a constant temperature of 13° C. The root tips were irradiated with X-rays at the same temperature. During the irradiation they were kept in a thin layer of water with air bubbled through it. The irradiation was performed at a voltage of 175 kilovolts. The X-rays were passed through a plate of window glass about 2 mm. thick and no other filtration was used. Two X-ray doses were applied: 600 r and 300 r, the first dose being given in 4.8 minutes and the second one in 2.4 minutes. The experimental conditions were exactly the same for the two species.

Fixations were made after 24 hours, 48 hours and so on every day during a period of 6 days. The root tips were fixed in MÜNTZING's modification of NAVASHIN's fixative (MÜNTZING, 1933, p. 131). They were embedded in paraffin and cut into longitudinal sections that were stained with crystal violet. The anaphases were examined and classified into normal and disturbed figures.

All cells that showed at least one chromosome bridge or at least one acentric fragment were classified as disturbed. Many cells, of course, showed both bridges and fragments, as these usually originate through the same change, *viz.* through an asymmetric interchange. No effort was made to score cells with fragments and cells with bridges separately, nor did we discriminate between the chromosome type of changes and the chromatid type of changes. Caution was used to eliminate cells that might have been damaged by the microtome knife. It is obvious that some changes must have escaped observation, *e. g.* dicentric anaphase chromosomes in which both the centromeres had moved to the same pole, and acentric fragments lying close to the anaphase groups. Due to errors of this kind the method of anaphase scoring is far from exact (*e. g.* WOLFF and LUIPPOLD, 1957), but it should be capable of giving some information in a study of the present kind.

It was our intention merely to find out which species of the two investigated showed the highest sensitivity without making any effort to get an exact quantitative estimation of the size of the difference in

TABLE 2. *Frequencies of normal and abnormal anaphases in two Hyacinthus species at various periods after irradiation with two doses of X-rays.*

X-ray dose	Days after irradiation	*H. orientalis*				*H. dalmaticus*			
		No. of cells	No. of normal cells	Abnormal cells		No. of cells	No. of normal cells	Abnormal cells	
				No.	%			No.	%
300 r	1	89	0	89	100	150	113	37	24.7
	2	138	3	135	97.8	105	81	24	22.9
	3	148	17	131	88.5	141	112	29	20.6
	4	107	4	103	96.3	117	108	9	7.7
	5	124	40	84	67.7	112	93	19	17.0
	6	117	58	59	50.4	—	—	—	—
600 r	1	—	—	—	—	127	68	59	46.5
	2	43	0	43	100	121	49	72	59.5
	3	142	5	137	96.5	120	63	57	47.5
	4	114	8	106	93.0	132	79	53	40.2
	5	157	25	132	84.1	123	89	34	27.6
	6	131	21	110	84.0	135	81	54	40.0

sensitivity. It seems probable that the methodical errors will be about the same for the two species and that for this reason a comparison is justified. In any case, we found a pronounced difference between the two species which could hardly be caused by a possible difference in size of the methodical errors between the two species.

The results are presented in Table 2 and in Figs. 4—5. One six-day-fixation of *H. dalmaticus* is missing because of shortage of roots on the bulb in question. There are no data for the one-day-fixation of the 600 r series of *H. orientalis* because of the absence of mitoses. This relatively strong X-ray dose had caused a mitosis-free period in this species. The mitodepression (reduced frequency of mitoses) was expressed also in the two-day-fixation, in which the frequency of mitoses was strongly reduced.

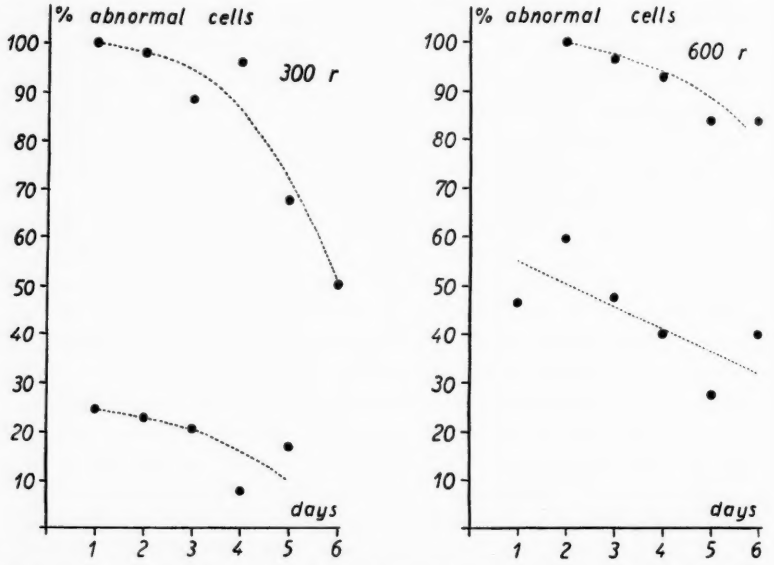
Our observations strongly suggest that the mitodepressive effect is more pronounced in the large chromosome species. Mitodepression is usually considered as a »physiological» effect of X-rays; »physiological» in this connection being used in contrast to such effects as chromosome breakage and mutation. There is, however, some evidence suggesting that the chromosomes may also be of great importance in this physiological effect. Thus, MARSHAK and BRADLEY (1944) found that poly-

ploids show a much smaller mitotic inhibition than diploids when treated with the same X-ray dose. They had the impression, however, that chromosome size had no influence in the case of mitotic inhibition. In this respect they confirmed an earlier observation by MARSHAK (1937). The dependence of the reduced frequency of mitoses in X-ray experiments on polyploidy and possibly on chromosome size opens interesting aspects that should be investigated further.

When making a comparison between the X-ray sensitivity of two different species, as was made here, it will scarcely be sufficient to compare the effects at a single fixation time. The effect will vary for different times of fixation, and as the mitotic cycles may be of different lengths in different species, it is quite possible that simultaneous fixations from the two species may not correspond to one another. For this reason it is advisable to make a whole series of fixations for both species in order to get a more complete picture of the time-effect curves. We made the first fixation 24 hours after the treatment, because we wanted to avoid the stickiness characteristic of the so-called primary effect, which is found in fixations made a short time after the irradiation. At the low temperature (13° C) used in our experiment the mitotic cycle is fairly slow in *H. orientalis*, at least. In this species the first root tip cells pass into the second division after the irradiation (X_2) on the fifth day after the treatment. The occurrence of X_2-mitoses is recognizable by the presence of chromosome fragments in the cytoplasm of prophase cells. Such cells are absent in the 4-day-fixation but common in the 5- and 6-day-fixations. Unfortunately, we have not been able to find out in the same way when *H. dalmaticus* is passing into its X_2-division. Because of the small chromosome size the degenerating chromosome fragments in the cytoplasm of the prophase cells are difficult to distinguish from other cytoplasmic bodies that also stain with crystal violet. We have, however, a strong suspicion that the mitotic cycle may be considerably shorter in *H. dalmaticus* than in *H. orientalis*.

There are good reasons to suspect that simultaneous fixations in the two species do not actually correspond to one another. Under these circumstances it is not appropriate to make a statistical test that is founded upon comparisons of simultaneous fixations. We tested the difference by comparing for each dose the lowest point on the upper curve with the highest point on the lower curve in Figs. 4—5. In both cases the χ^2-test (performed on the figures of Table 2) gave a p-value of less than 0.001.

Comparisons within each series of the frequencies of disturbed cells



Figs. 4—5. The frequencies of abnormal anaphases in *Hyacinthus orientalis* (the upper curve in both figures) and *H. dalmaticus* (the lower curve) at various periods after irradiation with 300 and 600 r units of X-rays. The lines are based on visual judgement only, and too much significance should not be ascribed to their details.

show a highly significant heterogeneity between different times of fixation. Undoubtedly, this heterogeneity is caused largely by a gradual increase in the frequency of the normal cells as time went on, which is due to two effects, at least. *Firstly*, the sensitivity of the resting stage chromosomes to X-ray fragmentation is higher in late resting stage than in early resting stage (*e. g.* KIHLMAN, 1955). *Secondly*, when the roots pass over to X_2 and later mitoses the frequency of disturbed cells is decreased, *partly* by selection, as the damaged cells have a weaker growth power than the normal ones, and *partly* because many of the damaged chromosomes have been mechanically lost at the previous mitosis.

The two species compared by us differed from one another not only in chromosome size but also in chromosome number. For this reason it might be appropriate to consider whether this latter difference might have a disturbing influence in our experiment. There are two possibilities:

(1) The increase in chromosome number may have originated with negligible duplication of chromosome material. This material may be practically the same in the two species although in one of them it involves some additional centromeres. In this case, the difference in chromosome number would have scarcely any influence on the radiosensitivity.

(2) The higher chromosome number in one of the two species might be connected with an appreciable duplication of chromosome material through some kind of polyploidy or secondary polyploidy. A difference of this kind could theoretically produce a disturbance in an experiment of the present kind. Polyploidy is known to cause an increased frequency of breaks per cell. As, however, our material with the higher chromosome number had actually fewer changes per cell, it can be concluded that the difference observed by us cannot originate from a disturbance of this kind.

The results of our experiment are obviously in agreement with the opinion that species having larger chromosomes show more fragmentation per cell than species with smaller chromosomes, when treated with the same X-ray dose. Naturally, these results should be confirmed on other species pairs of a similar kind before they can be generalized. The idea of the existence of a regularity of this kind is also supported by the results of MARSHAK (1937), who from his observations concluded that a larger chromosome size was accompanied by a higher degree of X-ray fragmentation. This opinion is, of course, also in good agreement with the observations by SPARROW and CHRISTENSEN (1953) and by NYBOM (1956) on the phenotypical damage and the sterility induced by gamma-radiation in materials differing in chromosome size.

Naturally, the fact that chromosome size is a factor of importance for the radiosensitivity of living organisms or for the breakability of their chromosomes does not exclude the possibility that other factors can also be very important in this connection. The existence of a variation in these reactions towards irradiation without a variation in chromosome size is a result which, of course, does not in any way conflict with the opinion that chromosome size is an important factor in this connection.

This study has been supported by a research grant from the Agricultural Research Council of Sweden, which is gratefully acknowledged. One of us (ROSALIND MORRIS) participated in the study while on leave from the University of Nebraska as a Guggenheim Fellow.

SUMMARY

Hyacinthus dalmaticus (*Bellevalia dalmatica*) has $2n=20$ chromosomes, which are much smaller than those of *Hyacinthus orientalis* ($2n=16$). The problem of differences in chromosome size between related species is discussed. When the two species are treated with the same X-ray dose, many more chromosome changes per cell are obtained in the big chromosome species.

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SUR L'ÉVOLUTION DES LÉSIONS CAUSÉES PAR LA 8-ETHOXYCAFÉINE CHEZ *HORDEUM SATIVUM* ET CHEZ *VICIA FABA*

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(Reçu le 26 août 1957)

I. INTRODUCTION

C'EST GOSSELIN qui, en 1940, a décrit pour la première fois, les effets de la caféine sur la cellule végétale chez l'Avoine et chez le Pois. Dans le règne animal, il faut citer les travaux de HAAS (1941) sur l'œuf d'Anodonte. En 1944, MANGENOT et CARPENTIER ont observé des effets mitoclasiques de cette substance chez l'Oignon et chez le Froment; citons aussi en 1948, les observations de DEYSSON chez l'Oignon également. L'action mitoclasique de la caféine fût encore démontrée par KIHLMAN et LEVAN en 1949 sur le même matériel. Les observations de MANGENOT et CARPENTIER mettaient en évidence des effets primaires de cette substance tels que la présence de ponts chromatiques en anaphase; celles de KIHLMAN et LEVAN montrent qu'à côté de ces effets primaires, on recontre également d'importants effets secondaires.

Depuis ces recherches, les effets de nombreuses bases puriques sur les chromosomes ont été décrits. KIHLMAN (1949, 1950, 1952) a montré l'activité pharmacodynamique des dérivés caféiniques substitués sur le carbone 8 dans la genèse des accidents radiomimétiques. A ce point de vue, la 8-Ethoxycaféine s'est révélée d'une efficacité remarquable. Une localisation élective des cassures chromosomiques a été attribuée à ce dérivé. C'est ainsi que les investigations de KIHLMAN et LEVAN (1951) montrent, chez *Vicia faba*, un nombre considérable de satellites séparés du chromosome satellitique et ces auteurs concluent à la localisation des

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lésions au niveau de la zone d'attache du satellite. Dans le cas de *Pisum sativum* chez lequel les constrictions satellitiques n'ont pas de rapport avec le nucléole (HÅKANSSON et LEVAN, 1942), KIHLMAN (1952) a démontré une même électivité dans l'action de la 8-Ethoxycaféine.

Chez *Hordeum sativum*, EHRENBERG, GUSTAFSSON et VON WETTSTEIN (1956) ont montré que la 8-Ethoxycaféine augmente considérablement la stérilité de la première génération sans accroître le taux de mutations. On serait donc en présence d'une substance capable de casser les chromosomes sans induire de mutations visibles. Etant donné l'importance que peut avoir en génétique, la production *ad libitum* de plantes à caryotypes modifiés, nous avons repris l'étude de l'évolution des lésions dues à l'E.O.C. comparativement chez *Hordeum sativum* et chez *Vicia faba*.

II. MATÉRIEL ET MÉTHODE

Nous avons utilisé des graines de *Hordeum sativum* de la variété Bonus et des graines de *Vicia faba* d'une variété commerciale (Åkerböna, Weibull).

La 8-Ethoxycaféine a été utilisée en solution à la dose de 0.15 gr.% pendant $1^1/_2$ heure et 6 heures, à la température de 20° C. Les graines sont trempées dans cette solution soit sèches, soit prégermées pendant 24 heures sur papier filtre humecté par l'eau de la distribution. Après traitement, les graines sont lavées à l'eau courante et sèchées sommairement avant l'ensemencement en boîtes de Petri, sur papier filtre, à 20° C.

Les investigations portent sur les pointes radiculaires des racines principales chez *Vicia* et des 5 premières racines chez *Hordeum*, aucune fluctuation statistiquement importante n'ayant été enregistrée entre les différentes racines d'une même plante. Les pointes radiculaires sont fixées 35 h., 75 h., 125 h., 200 h., 250 h. et 350 h. après le début de la mise en germination (fin du traitement). Chez *Hordeum*, le temps de 35 h. est celui qui paraît correspondre au maximum d'expression des effets primaires et celui de 75 h. au début de l'apparition des micronoyaux prophasiques chez *Hordeum* comme chez *Vicia*. Bien que la désynchronisation mitotique ne soit pas ultérieurement la même, les prélèvements ont été effectués pour les deux plantes, après les mêmes temps en vue de la comparaison. Les fixations ont été réalisées dans le fixateur de Carnoy pendant 24 h. maximum et les racines ont été observées après Feulgen »squashes».

III. RÉSULTATS

A. Lésions primaires ou effets physiologiques

Chez *Hordeum*, la première mitose après la fin du traitement nous a permis d'observer l'apparition des classiques lésions primaires dues au toxique:

en métaphase: »clumping» et érosion chromosomique,

en anaphase: »stickiness», érosion chromosomique, sticky-bridges et fragmentation primaire due au stickiness.

L'érosion chromosomique se manifeste d'une manière inconstante. Tantôt, elle se limite à quelques zones achromatiques visibles aussi bien en métaphase qu'en anaphase; d'autres fois, elle est tout à fait généralisée et donne aux figures mitotiques, un aspect en quelque sorte pulvérisé.

Chez *Vicia*, par contre, les observations effectuées après 35 h. ne révèlent que des fragmentations en méta- et en anaphase. Les chromosomes ne se présentent jamais sous l'aspect observé chez *Hordeum*. On doit donc admettre que si ces lésions existent, elles sont ici beaucoup plus labiles, ayant à ce moment, entièrement disparu. Des pseudochiasmas (LEVAN et TJIO, 1948) ont été observés mais sont d'occurrence très rare pour les deux espèces. Aucun autre effet physiologique que ceux mentionnés ci-dessus n'a été relevé aux doses d'E.O.C. utilisées.

Le tableau I résume les différences de comportement entre les deux plantes lors de la mitose X_1 (Première mitose consécutive au traitement). Après 75 h., la disparition des effets primaires chez *Hordeum* et la

TABLEAU I. *Différences observées dans l'apparition des lésions primaires (mitoses X_1) chez Hordeum sativum et chez Vicia faba.*

Espèces	Temps de traitement	Prégermination	% de métaph. perturbées	% d'anaphases perturbées	N. de fragments métaph./cell.	N. de fragments anaph./cell.	N. de ponts/anaphase
Hordeum sativum	1 1/2 h.	—	94.6	37.8	0.01	—	0.15
		+	93.7	55.5	0.18	0.05	0.22
	6 h.	—	92.8	36.5	0.16	0.03	0.046
		+	94.7	57.0	0.11	0.02	0.06
Vicia faba	1 1/2 h.	—	—	—	0.01	0.018	—
		+	—	—	—	—	—
	6 h.	—	—	—	0.13	0.03	0.14
		+	—	—	—	—	—

corrélation existant entre cette disparition et l'apparition des micronoyaux dans les tissus méristématiques nous paraît pouvoir réellement servir de test à un jalonnement d'une succession mitotique perturbée par le toxique.

B. La répartition des cassures dans le génome

L'apparition et le maintien des cassures ont été observés pour les deux espèces au cours des mitoses successives jusqu'à 350 h. après la fin du traitement. Nous avons négligé de tenir compte des fragments primaires en raison du fait que même dans les conditions les plus favorables, les cassures primaires sont impossibles à localiser avec certitude. Le tableau II résume l'évolution des cassures chez *Vicia* et leur répartition, dans les différentes zones décelables du génome. L'étude a été réalisée pour les deux temps d'action de l'E.O.C., avec et sans prégermination préalable au traitement.

Le tableau II montre que:

1) chez *Vicia*, confirmant les données de KIHLMAN et LEVAN, le taux de cassures isolant les satellites est très élevé;

2) le taux de cassures centromériques est élevé relativement au taux de cassures localisées aux régions euchromatiques;

3) le taux de cassures euchromatiques est beaucoup plus faible dans le chromosome (M) que dans les chromosomes (st);

4) après 6 heures de traitement, le taux de cassures ainsi que leur répartition dans le génome sont les mêmes avec ou sans prégermination;

5) après $1^1/_2$ heure de traitement, le taux de cassures est plus élevé sans prégermination qu'avec prégermination mais le taux relatif de satellites isolés est moins fort.

Remarquons que, dans le tableau II, il a été tenu compte uniquement des casssures »isoloci» à l'exclusion des cassures chromatidiques (=cassures B′ de DARLINGTON) toujours en nombre très peu élevé et pratiquement négligeable dans nos conditions d'expérience.

Quels sont, à ce sujet, les résultats de nos observations chez *Hordeum*? Chez celui-ci, il est possible d'identifier les constrictions secondaires des chromosomes décrits sons le nom de chromosome VI et de chromosome VII (TJIO et LEVAN, 1950) ainsi que, dans la variété Bonus, les constrictions tertiaires parfois peu apparentes cependant des chromosomes I et VII.

Le tableau III rend compte de la répartition des lésions dans le génome de *Hordeum*.

TABLEAU II. *Evolution des cassures chromosomiques chez Vicia faba et leur répartition dans le génome (nombre de cassures par métaphase).*

Durée du traitement	Temps après le traitement	Cassures dans la constriction nucléolaire	Cassures dans le centromère du chromosome M	Cassures dans les centromères des chromosomes St	Cassures dans l'euchromatine du chromosome M	Cassures dans l'euchromatine des chromosomes St
A. Sans prégermination						
1 1/2 h.	75 h.	0.04	—	—	—	0.02
	125 h.	0.05	—	—	—	0.01
	200 h.	0.09	0.03	0.03	—	0.08
	250 h.	0.21	—	—	—	0.06
	350 h.	0.24	—	—	—	0.09
6 h.	75 h.	0.03	—	—	—	0.01
	125 h.	0.04	0.04	0.01	—	0.02
	200 h.	0.21	0.02	—	—	0.09
	250 h.	0.22	0.01	0.01	0.02	0.06
	350 h.	0.10	—	—	—	0.04
B. Avec prégermination						
1 1/2 h.	75 h.	—	—	—	—	—
	125 h.	—	—	0.01	—	0.02
	200 h.	0.01	—	0.01	—	0.03
	250 h.	0.27	0.02	0.01	0.02	0.03
	350 h.	0.10	—	—	—	0.02
6 h.	75 h.	0.04	—	0.01	—	0.01
	125 h.	0.02	—	—	—	0.01
	200 h.	0.12	—	—	0.01	0.08
	250 h.	0.22	0.01	0.03	0.01	0.07
	350 h.	0.23	0.02	—	—	0.09

TABLEAU III. *Evolution des cassures chromosomiques chez Hordeum sativum et répartition dans le génome (nombre de cassures par métaphase).*

	350 h.	0.23	0.02	—	—	0.09			

TABLEAU III. *Evolution des cassures chromosomiques chez Hordeum sativum et répartition dans le génome (nombre de cassures par métaphase).*

Durée du traitement	Temps après le traitement	Constrictions primaires			Constrictions secondaires			Constr. tertiaires		Cassures euchrom. de tous les chrom.
		Chrom. VI	Chrom. VII	Autres chrom.	Chrom. VI	Chrom. VII	Chrom. non identif.	Chrom. I	Chrom. VII	
A. Sans prégermination										
1 1/2 h.	35 h.	—	—	—	—	—	—	—	—	—
	75 h.	0.03	0.02	—	0.07	0.02	—	—	—	0.12
	125 h.	—	—	—	0.08	0.02	—	0.06	—	0.11
	200 h.	—	—	—	—	—	—	—	—	—
	250 h.	—	—	—	—	—	—	—	—	—
	350 h.	—	—	—	—	—	—	—	—	—
6 h.	35 h.	—	—	—	—	—	—	—	—	—
	75 h.	0.02	0.01	0.01	0.08	0.03	0.02	—	—	0.11
	125 h.	—	—	0.05	0.02	0.02	0.01	0.01	—	0.04
	200 h.	—	—	—	—	—	—	—	—	0.05
	250 h.	—	—	—	—	—	—	—	—	—
	350 h.	—	—	—	—	—	—	—	—	—
B. Avec prégermination										
1 1/2 h.	35 h.	—	—	—	—	—	—	—	—	—
	75 h.	—	—	—	0.01	—	—	—	—	0.01
	125 h.	—	—	—	0.07	0.02	—	—	—	0.06
	200 h.	—	0.02	0.01	0.01	0.02	0.02	—	—	0.01
	250 h.	—	—	—	—	—	—	—	—	—
	350 h.	—	—	—	—	—	—	—	—	—
6 h.	35 h.	—	—	—	—	—	—	—	—	—
	75 h.	0.01	—	—	0.01	—	0.01	—	—	0.01
	125 h.	0.02	—	0.02	0.06	0.04	0.02	0.02	—	0.06
	200 h.	—	0.02	—	0.08	0.02	0.03	0.01	0.02	0.02
	250 h.	—	—	—	—	—	—	—	—	—
	350 h.	—	—	—	—	—	—	—	—	—

Le tableau III montre que

1) il y a une forte concentration des lésions au niveau des constrictions secondaires séparant ainsi les satellites des chromosomes VI et VII. Le satellite du chromosome VI est plus fréquemment isolé que celui du chromosome VII.

2) il y a un nombre élevé de cassures au niveau des centromères et des constrictions tertiaires.

3) le rapport $\frac{\text{n*. de cassures dans les constrictions}}{\text{n. de cassures dans l'euchromatine}} =$

$K_1 \frac{30}{23} = 1.3$ ($1^1/_2$ h. sans prég.) $K_3 \frac{18}{8} = 2.25$ ($1^1/_2$ h. avec prég.)

$K_2 \frac{28}{20} = 1.4$ (6 h. sans prég.) $K_4 \frac{39}{9} = 4.33$ (6 h. avec prég.)

(les fragments pseudo-satellitiques ont été soigneusement écartés).
De ce rapport, on déduit que

a) sans prégermination, les taux de lésions sont du même ordre de grandeur pour les 2 temps d'action du toxique. ($P = 0.50 < 0.70$).

b) avec prégermination, le taux total de lésions est moins élevé pour $1^1/_2$ h. d'action que pour 6 h. mais le taux relatif de lésions localisées en dehors de l'euchromatine est accru. ($P = 0.01 < 0.02$).

Comme pour *Vicia*, il n'a été tenu compte, dans le tableau III, que des cassures isoloci pour la raison exposée antérieurement.

Devant cette localisation spécifique des lésions, il est d'un grand intérêt de pouvoir disposer d'éléments de comparaison avec d'autres agents mutagènes. Disons que des essais réalisés avec plusieurs drogues connues pour leur aptitude à casser les chromosomes (éthylène-oxyde, bis-epoxybutane, éthylène-imine) ne nous ont jamais donné cette répartition lésionnelle pas plus que les radiations ionisantes.

A titre de comparaison, nous figurons (Schéma I) la répartition de 50 cassures prises au hasard dans les chromosomes satellitiques (VI et VII) de *Hordeum*. Etant donné l'évidente répartition des cassures dans les constrictions de ces chromosomes à la suite de l'action de la 8-Ethoxycaféine comparée à la répartition au hasard des cassures dues aux rayons X (24 kr.), nous avons pensé qu'il n'était pas nécessaire de traiter le problème d'une manière statistique plus poussée.

Comparons maintenant la répartition et l'évolution des cassures chez *Hordeum* et chez *Vicia*.

a) Chez les deux espèces, il existe une localisation systématisée des cassures ce qui nous paraît être un argument décisif en faveur de la

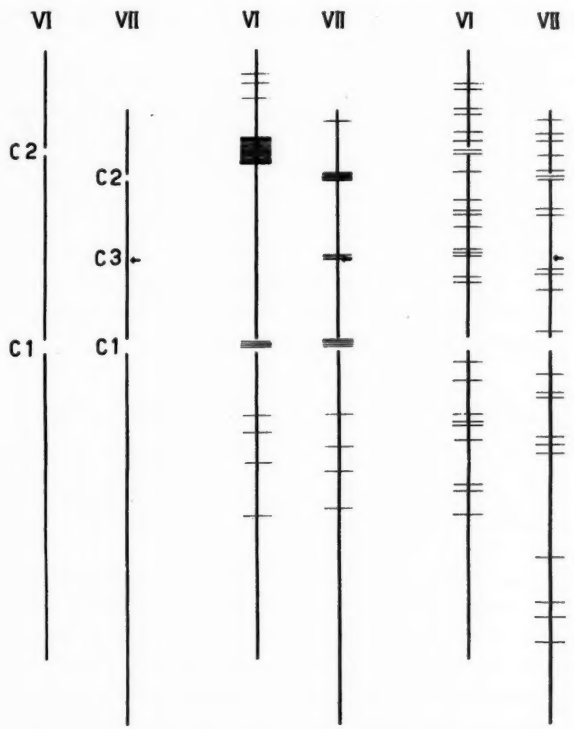

Schéma I. — Répartition des cassures provoquées par l'E.O.C., 0.15 % (au centre) et par les rayons X, 24 Kr (à droite) dans les chromosomes VI et VII de *Hordeum sativum* (sur 50 cassures dans chaque cas). A gauche, chromosomes VI et VII avec leurs constrictions primaires (C_1), secondaires (C_2) et tertiaire (C_3).

spécificité de l'action de cette substance. Le taux de cassures isolant les satellites est cependant beaucoup plus élevé chez *Vicia* que chez *Hordeum* compte tenu du fait que de telles cassures puissent exister spontanément chez *Vicia* (LEVAN et LOTFY, 1950). Dans nos conditions expérimentales, aucun satellite isolé ne fut observé chez les témoins de *Vicia* ou de *Hordeum*. Chez *Vicia*, le taux de cassures localisées au niveau centromérique est relativement moindre que chez *Hordeum*. Dans ce cas-ci, nous avons donc une répartition des cassures rappelant celle décrite par KIHLMAN (1952) chez *Pisum*. De l'examen de la localisation des cassures, il apparaît donc qu'aucun argument d'ordre cyto-

logique en faveur ou contre l'hypothèse nucléolaire, ne peut être retenu tendant à expliquer l'action des purines.

b) Une différence très marquée a été constatée dans l'évolution des cassures chez les deux espèces. Chez *Hordeum*, aucun fragment n'est visible 250 heures après le traitement et ce fait se confirme à 350 heures. Chez *Vicia*, le nombre de fragments augmente au cours des mitoses successives jusqu'à la 250e heure. Après ce temps, il tend à se stabiliser.

c) Il semble que chez *Hordeum*, le temps d'action de l'E.O.C. pendant $1^1/_2$ heure sans prégermination, soit suffisant pour atteindre un seuil d'efficacité, le taux de cassures étant du même ordre de grandeur après 6 heures. Il n'en est pas de même chez *Vicia* où le taux de cassures s'accroît quand on augmente le temps d'action.

C. L'évolution des réunions chromosomiques

Le tableau II nous a montré que les cassures spécifiquement localisées ne diminuent pas en nombre chez *Vicia* pendant plusieurs mitoses. Devant un tel état de choses, il est nécessaire de tenter de répondre à plusieurs questions.

1) Les cassures observées au cours des mitoses successives sont-elles produites secondairement ou bien s'agit-il de fragments susceptibles de se maintenir? 2) Y a-t-il un moment où les fragments disparaissent ou bien peuvent-ils être engendrés indéfiniment? 3) A quel type de réunions les cassures spécifiques peuvent-elles donner naissance? 4) L'évolution des réunions est-elle la même chez *Hordeum* que chez *Vicia*?

Le tableau III nous a montré que chez *Hordeum*, aucune cassure n'a persisté après 250 heures. Les réunions chromosomiques sont très malaisées à discerner chez *Hordeum*, en métaphase. Nous avons alors tenté de les décrire pendant l'évolution mitotique de *Vicia*.

Le tableau IV résume l'évolution de différents types de réunions pendant toute la période de 350 heures consécutives au traitement. Nous n'avons figuré que les réunions qui ont pu être localisées à l'exclusion d'autres types tels que les réunions entre chromatides-sœurs. Après avoir observé que le taux de réunions est supérieur après 6 heures qu'après $1^1/_2$ heure, nous avons choisi ce dernier traitement en vue d'analyser l'évolution des différents types de réunions.

Nous avons constaté une profusion de figures de réunions étonnantes consistant en configurations »polyradiales» spécifiquement localisées au niveau des zones où les cassures ont été notées en fréquence élevée. Nous distinguons ces configurations polyradiales des configurations tri-

TABLEAU IV. *Analyse et évolution de différents types de réunions chromosomiques chez Vicia faba. Interchangements chromatidiques par métaphase.*

Traitement	Temps après le traitement	Interchangements chromatidiques localisés dans les centromères et dans la constriction nucléolaire										Interchangements chromatidiques localisés dans l'eu-et l'hétérochromatine			
		MM	mm	Mm	M St	M St St	M St	m St St	St St	St St St	4 St	M	Satell	St	St M
6 h. à sec.	35	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	75	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	125	0.010	0.010	—	—	—	—	—	—	0.011	—	—	—	—	—
	200	0.022	—	—	—	0.022	—	—	0.021	0.034	—	—	0.020	—	—
	250	0.010	0.010	0.029	0.100	0.030	0.021	—	0.130	0.080	0.019	0.009	0.020	0.059	0.022
	350	—	—	—	0.010	—	—	—	0.040	0.010	0.020	0.010	—	0.020	—
6 h. avec prégermination pendant 24 h.	35	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	75	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	125	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	200	—	—	—	0.010	—	—	—	0.080	0.011	—	—	0.011	0.022	0.020
	250	0.017	—	0.010	0.020	0.01	0.016	0.011	0.100	0.010	—	0.012	0.010	0.010	0.030
	350	0.015	—	—	0.030	0.02	—	—	0.110	0.040	0.010	—	—	—	—

Légende

Pour les localisations en dehors de l'eu-et de l'hétérochromatine	M désigne le centromère du chromosome M m » l'organisateur nucléolaire St » le centromère des chromosomes St
Pour les localisations dans l'eu-et l'hétérochromatine	M » » chromosome M Satell. désigne le satellite St désigne les chromosomes St

radiales habituelles dans l'euchromatine car elles réunissent en un ou plusieurs points voisins, plus de 4 chromatides (jusqu'à 8!). Ces figures sont parfois très difficiles à distinguer avec certitude car elles nécessitent des métaphases parfaitement étalées. Il s'agit donc de réunions de 2 ou de plusieurs chromosomes à un même niveau spécifique (telles que celles des Fig. 3 et 4, pl. II). Nous avons aussi observé quelques typiques configurations triradiales dans l'euchromatine au sens où l'on comprend généralement ces figures mais celles-ci sont comparativement beaucoup plus rares.

Les planches I et II donnent, l'une ou l'autre conséquence de ces figures au cours de l'évolution mitotique de *Vicia*. Ces figures seront interprétées ultérieurement.

Le tableau IV montre que

1) aucun interchangement chromatidique n'est observé avant 125 heures après la fin du traitement (compte non tenu des réunions chromatidiques B′, cfr. plus haut, possibles mais certainement rares).

2) les fréquences de réunions augmentent pour atteindre un maximum 250 heures après la fin du traitement.

3) les répartitions des réunions dans le génome sont les mêmes qu'il y ait eu ou non prégermination des graines avant le traitement.

4) il existe une fréquence supérieure de configurations polyradiales dans les centromères et dans la constriction nucléolaire comparativement aux configurations triradiales localisées en d'autres lieux du génome.

5) la plus grande fréquence de configurations polyradiales implique les centromères de 2 chromosomes différents et, dans un grand nombre de cas, le centromère du chromosome M.

Nous devons insister sur le fait qu'aucune image complexe de réunions n'a été rencontrée chez *Hordeum*. Les configurations triradiales dans l'euchromatine sont rarissimes et nous n'avons jamais observé aucune configuration polyradiale au niveau des constrictions.

L'évolution de ces types de réunions nous a montré la possibilité d'obtenir un génome de *Vicia* sans satellite. Etant donné la fréquence de lésions au niveau de l'organisateur nucléolaire, on peut se demander si de telles cellules peuvent survivre ou si elles sont nécessairement vouées à la mort à brève échéance. On sait que toute cassure isolant le bras distal du chromosome M peut engendrer un micronoyau nucléolé. Mc LEISH (1954) a observé qu'avec l'hydrazide maléique, il existe un synchronisme dans la division de ces micronoyaux nucléolés et des noyaux-frères anucléolés. Il nous a paru intéressant de mettre l'évolution des micronoyaux en relation avec l'évolution des fragments.

TABLEAU V. *Comparaison de l'évolution des taux de satellites isolés et de micronoyaux chez Vicia faba.*
(*24 heures de prégermination, 6 heures de traitement.*)

Temps après le traitement	Taux de satellites séparés pour 100 métaphases	σ	Taux de métaphases pour 100 cellules	σ	Taux de satellites séparés pour 100 cellules	σ	Taux de micronoyaux pour 100 cellules	Pourcentage de ces micronoyaux nucléolés	Taux de micronoyaux nucléolés pour 100 cellules	σ
35 h.	—	—	—	—	—	—	—	—	—	—
75 h.	4	± 0.0044	1.8	± 0.014	0.072	± 0.0044	4.8	13.1	0.6288	± 0.091
125 h.	2	± 0.014	2.6	± 0.0312	0.052	± 0.014	15.3	27.7	4.2381	± 0.554
200 h.	12	± 0.0312	2.7	± 0.0704	0.324	± 0.0312	8	33.3	2.6640	± 0.466
250 h.	22	± 0.0704	3.6	± 0.0805	0.792	± 0.0704	2	45	0.900	± 0.392
350 h.	23	± 0.0805	5.4	± 0.1265	1.242	± 0.0805	0.7	48	0.284	± 0.126

Le tableau V résume les résultats de cette comparaison pour un traitement particulièrement efficace dans la production de fragments et de micronoyaux (prégermination pendant 24 h. et traitement pendant 6 h.).

Le tableau V indique que le pourcentage relatif de micronoyaux nucléolés par rapport au pourcentage de micronoyaux anucléolés augmente au cours des mitoses successives jusqu'à 350 heures mais le taux de micronoyaux nucléolés par cellule n'augmente que jusqu'à la 125^{e} heure puis diminue ensuite alors que le taux de fragments satellitiques continue d'augmenter. Il faut donc admettre qu'après 125 h., les satellites isolés ne donnent plus naissance à des micronoyaux nucléolés et que, dès lors, ils sont privés d'organisateur nucléolaire, celui-ci restant attaché au bras proximal du chromosome M. Il serait donc possible d'engendrer de cette manière, un génome sans satellite mais avec organisateur nucléolaire.

Si l'on compare les données des tableaux II, IV et V, on voit donc que, concernant les satellites,

a) le taux de satellites isolés augmente au cours des mitoses successives,

b) le taux de réunions et particulièrement d'interchangements chromatidiques affectant la constriction nucléolaire augmente également alors que

c) le nombre de micronoyaux nucléolés diminue quand le taux de réunions devient maximum (250 h.).

Devant cet état de choses, il faut donc admettre que

1) les cassures au niveau de la constriction nucléolaire peuvent être engendrées secondairement au cours de l'évolution mitotique,

2) ce fait peut expliquer l'augmentation du taux de satellites séparés au cours de l'évolution mitotique,

3) les cassures secondaires séparant les satellites doivent se faire électivement au point de jonction du satellite et de la constriction nucléolaire.

Il était dès lors, intéressant d'envisager l'évolution des taux de réunions entre chromatides-sœurs (sister-reunions SR de DARLINGTON) dans les satellites isolés. Cependant, ce taux de réunions ne variant pas nettement au cours de l'évolution ne nous a pas paru pouvoir être retenu.

Nous interprétons donc les figures des planches I et II de la manière suivante.

Interprétation des figures

Chez *Vicia*, il est logique de considérer le taux d'interchangements chromatidiques isoloci spécifiquement localisés comme un corollaire du taux très élevé de cassures localisées dans les zones identiques. KIHLMAN (1955) a observé que le taux d'interchangements chromatidiques excède largement le taux de cassures dans le cas de *Vicia* alors que dans le cas d'*Allium cepa*, la proportion est inverse.

Nos observations satisfont pleinement aux précédentes pour les deux espèces envisagées ici. Nous ne pouvons, en effet, que faire ressortir le contraste existant entre elles au point de vue de ces réunions. Insistons sur l'aptitude des chromosomes de *Vicia* à subir des translocations homologues en des points homologues, cette aptitude s'estimant au nombre très élevé de configurations »polyradiales». Les photos 3 et 4, planche II, donnent des exemples de telles réunions. L'absence de ces figures chez *Hordeum* montre que, chez celui-ci, il n'y a aucune tendance analogue et que, si des translocations induites aux niveaux spécifiques existent, elles ne pourraient consister qu'en translocations de bras chromosomiques entiers. Nous n'avons cependant jamais mis en évidence ces translocations avec certitude. Une investigation en première génération serait pour celà nécessaire.

Un des faits saillants dans l'évolution des réunions chez *Vicia* est la genèse de chromosomes M »polysatellitiques». Certes, la présence de configurations polyradiales au niveau de la constriction nucléolaire pourrait seule suffire à expliquer que des fragments divers restent appendus au niveau de la même constriction mais il a été presqu'exclusivement relevé que les fragments appendus à une seule constriction nucléolaire sont des satellites. A partir de ce fait, il est possible d'imaginer une autre séquence expliquant la formation de ces curieuses figures. (Schéma II).

a) Il se formerait tout d'abord une configuration tétraradiale consécutive à 2 cassures isoloci en des points homologues dans la constriction nucléolaire.

b) Les réunions pourraient se faire suivant le type 3, 3′ ou 3″.

En anaphase, le type 3 isolerait 2 satellites séparés par une constriction nucléolaire. Les figures 1 et 6, planche I s'expliqueraient de cette façon. Dans la figure 1, on observe, en métaphase, un anneau fait de 4 satellites ayant subi 2 réunions entre chromatides-sœurs et nettement séparés par une zone qui doit correspondre à un organisateur nucléolaire. Dans la figure 6, 2 satellites se suivant bout à bout, sont séparés

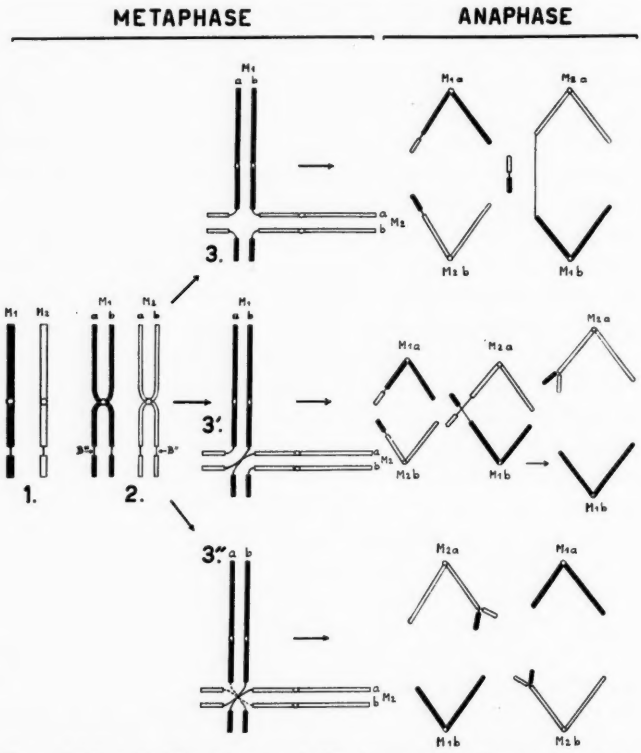

Schéma II. — Schéma de l'évolution probable des configurations polyradiales ayant lieu dans la constriction nucléolaire chez *Vicia faba* (cfr. explication dans le texte).

par la constriction. Les 2 chromosomes M forment un pont, séparés à un niveau qui paraît bien être également celui de l'organisateur. Par contre, dans la figure 8, les satellites sont isolés dans le cytoplasme et ne sont pas unis.

Les types 3′ et 3″ expliqueraient la formation de chromosomes M bisatellitiques en anaphase. Les figures 4 et 5, planche I et la figure 2, planche II s'expliquent de cette manière. Dans la figure 4, planche I, un satellite a disparu, les 2 chromosomes M forment un pont au niveau de l'organisateur. Dans la figure 5, planche I, 2 satellites sont appendus au même organisateur nucléolaire au pôle inférieur. Le pôle supérieur contient un chromosome M sans satellite. Les 2 pôles contenant un chromosome M normal, la figure satisfait au schéma 3′.

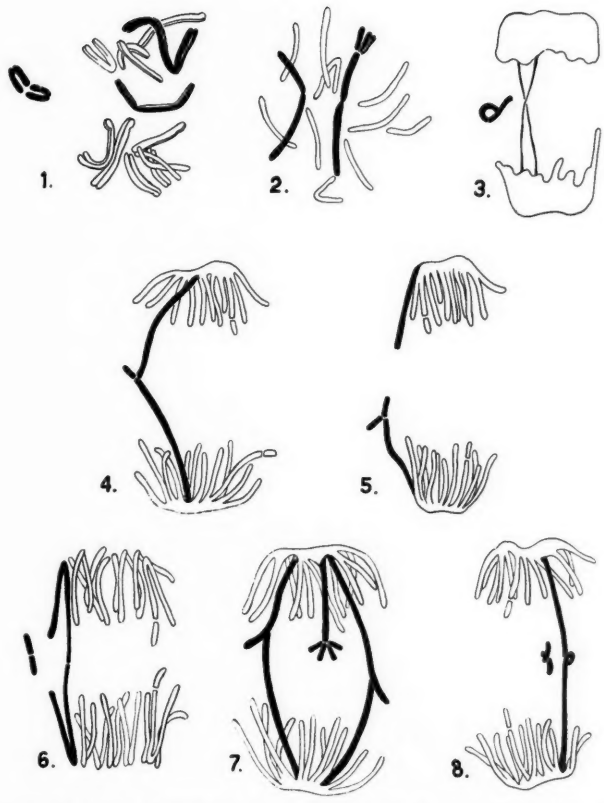

Planche I. — Quelques aberrations du chromosome M chez *Vicia faba* traité par l'E.O.C. — Fig. 1. Métaphase avec satellites isolés (en noir) et réunion formant un anneau. Les 2 chromosomes M sont privés de satellites. — Fig. 2. Métaphase avec, en noir, un chromosome M polysatellitique et un chromosome M privé de satellite. — Fig. 3. Anaphase avec double pont croisé (criss-cross) et fragment de chromosome M isolé. — Fig. 4. Anaphase avec pont formé par la réunion de 2 chromosomes M au niveau de l'organisateur nucléolaire. — Fig. 5. Anaphase avec pont cassé; au pôle inférieur, chromosome M bisatellitique. — Fig. 6. Anaphase avec pont formé par 2 chromosomes M et 2 satellites isolés réunis. — Fig. 7. Anaphase avec 2 ponts entre chromosomes M et chromosome M tétrasatellitique. — Fig. 8. Anaphase avec pont et satellites isolés.

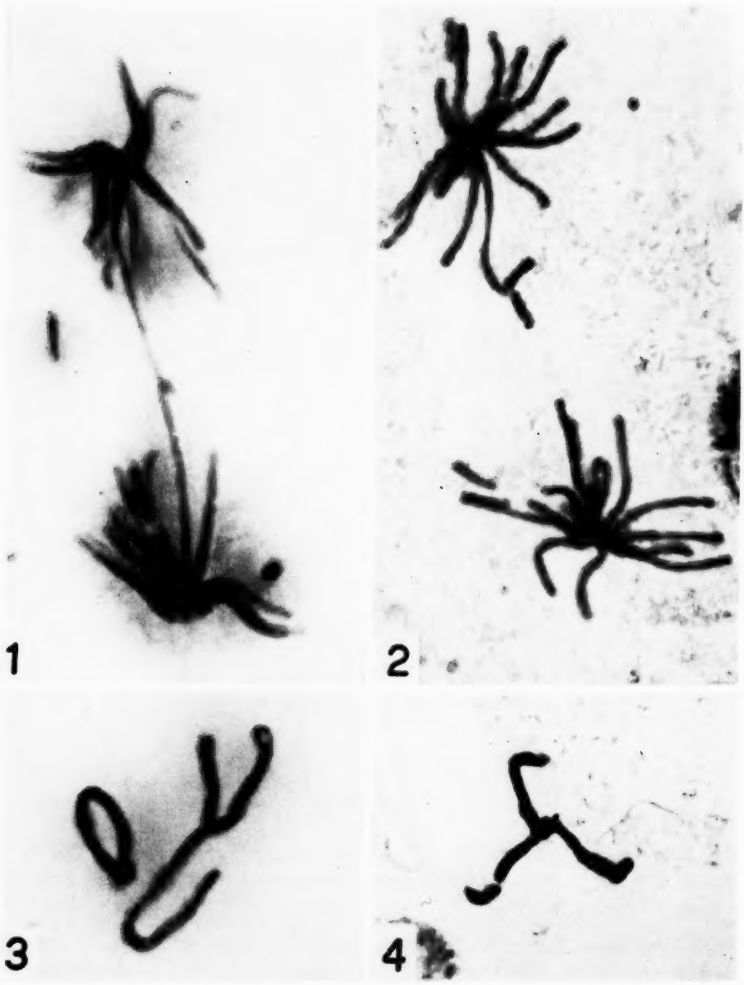

Planche II. — Fig. 1. Anaphase avec pont et satellite séparé du chromosome M. (EOC 0.15 %/trait. à sec/pdt 6 h./à 20° C/après 250 h./ ×1.800). — Fig. 2. Anaphase montrant 2 satellites attachés au même organisateur nucléolaire. (EOC 0.15 %/trait. à sec/pdt 6 h./à 20° C./après 250 h./ ×1.800). — Fig. 3. Configuration tétraradiale dans les centromères d'un chromosome S et d'un chromosome M ayant perdu son satellite. (EOC 0.15 %/trait. à sec/pdt 6 h./à 20° C/après 250 h.) ×1.800.). — Fig. 4. Configuration tétraradiale (chromosome M et S réunis par leur centromère). (EOC 0.15 %/trait. à sec/pdt 6 h./à 20° C/après 250 h.) ×1.600).

L'anaphase 2, planche II, montre aussi au pôle supérieur, 2 satellites au même organisateur nucléolaire et un autre chromosome M avec son satellite (au-dessus); le pôle inférieur contient un chromosome M avec son satellite (au-dessus, à gauche); sous ce satellite, un chromosome recourbé peut donner l'illusion, sur cette photo, d'un chromosome M satellitique.

c) Ces schémas évolutifs n'expliquent rien de ce qui se passe dans l'organisateur nucléolaire à l'échelle submicroscopique et ne présument en rien du fait que les cassures isoloci soient le premier phénomène se produisant sans contact préalable. Cependant, le fait que l'on observe de tels chromosomes bifides (»branched chromosomes» des auteurs anglo-saxons) plaiderait plutôt en faveur d'un contact intime se maintenant dans les zones où ont eu lieu les configurations polyradiales. On ne peut néanmoins trancher la question de savoir si ce contact consiste en une simple torsion, une liaison sub-chromatidique ou bien avec pontage généralisé dans la zone des constrictions.

Rappelons qu'un cas d'attachement d'un fragment chromosomique près du centromère a été relaté par LEVAN chez *Allium Allegheniense* et que l'existence de chromosomes bifides a été différentes fois prévue par divers généticiens. Nous n'avons pu enfin décider du fait de savoir si les figures à 4 satellites peuvent être formées directement (à partir du schéma 3″) ou bien secondairement par cassures et réunions de chromosomes déjà bisatellitiques. Néanmoins, comme le montrent les figures 2 et 7, planche I et la figure 1, planche II, la présence de chromosomes M tri- et tétrasatellitiques est un fait d'observation et une preuve supplémentaire en faveur de la localisation spécifique des lésions dues à la 8-Ethoxycaféine.

IV. CONCLUSIONS

L'examen de l'évolution des lésions dues à l'action de la 8-Ethoxycaféine permet de faire ressortir quelques analogies et quelques différences existant dans l'action de cette substance chez *Hordeum sativum* et chez *Vicia faba.*

(1) Concernant les lésions primaires: stickiness et érosion chromosomique, l'effet est beaucoup plus accusé chez *Hordeum* que chez *Vicia.* Pour cette dernière, l'effet primaire nous a paru labile.

(2) Le taux de cassures observé est plus élevé chez *Vicia* que chez *Hordeum* durant toute la période où les cassures ont été observées.

(3) Pour les deux espèces, il existe une spécificité d'action. Chez

Hordeum, un pourcentage important de cassures est localisé au niveau des centromères, des constrictions secondaires (chromosomes 6 et 7) et des constrictions tertiaires (chromosome 1 et 7). Chez *Vicia*, un pourcentage très élevé de cassures est localisé au niveau de la constriction nucléolaire et des centromères.

(4) Chez *Hordeum*, le taux de cassures diminue progressivement; 250 heures après le traitement, aucun fragment n'est observé. Chez *Vicia*, par contre, le taux de cassures électivement localisées au chromosome nucléolaire s'accroît au cours du développement. (Observations jusqu'à 350 heures.)

(5) Dans le cas de *Vicia*, il s'agit de cassures produites secondairement au cours de l'évolution mitotique à un niveau électif probablement situé au point de jonction du satellite et de la constriction nucléolaire.

(6) L'évolution lésionnelle laisse entrevoir la possibilité de maintenir des caryotypes fortement modifiés quoique viables.

Nous tenons à remercier tout particulièrement Monsieur le Professeur Ä. GUSTAFSSON qui fut le promoteur de cette recherche, pour ses conseils avisés et pour son exceptionnelle hospitalité. Nous remercions aussi vivement M. le Docteur B. KIHLMAN, qui a gracieusement mis à notre disposition la quantité d'E.O.C. nécessaire aux expériences. Nous remercions enfin l'Institut Interuniversitaire des Sciences nucléaires et la Fondation Universitaire de Belgique grâce auxquels nous avons pu effectuer un long séjour au Département de Génétique de l'Institut de Recherche forestière à Stockholm ainsi que la »Knut och Alice Wallenbergs Stiftelse» qui nous a donné toutes les facilités dans l'exécution de nos travaux.

SUMMARY

In order to investigate similarities and differences existing in the behavior of *Hordeum sativum* and *Vicia faba* treated with 8-E.O.C., we used this substance at the dose of 0.15 g % during $1^1/_2$ and 6 hours respectively on dry and 24 hours presoaked seeds. During the observed period of mitotic cycles from 35 hours to 350 hours after the treatment we have found that

(1) the physiological or primary effects are much more marked in *Hordeum* than in *Vicia* but, on the contrary, that the frequency of breaks is higher in *Vicia* than in *Hordeum*,

(2) a high specificity in the localisation of breaks, previously described for *Vicia*, was found in *Hordeum* too; here a high proportion of breakage in the secondary, primary and tertiary constrictions of the genome was revealed,

(3) fragments cannot be observed in *Hordeum* 200 hours after the end of treatment; in contrast, the number of specific fragments increases in *Vicia* up to 250 hours,

(4) for this species only and not for *Hordeum*, we found a proportion of configurations involving specially localised breaks. Those astonishing configurations, denoted as »polyradial», give rise to secondary breaks and may lead to profound modifications of the genome, such as »polysatellitic» chromosomes.

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CORRELATION STUDIES BETWEEN THE PARENTS AND THE PROGENY GENERATIONS IN OIL FLAX

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(Received September 13th, 1957)

IN the course of a more extensive investigation on oil flax (MANNER, 1956) an opportunity presented itself of carrying out a study on the correlation between the parents, the difference between them, the difference between F_2 and the parents, the difference between F_4 and the parents, and the difference F_2—F_4. The present author has studied in the above-mentioned paper the individual crosses and found them to differ from each other with regard to the studied characters. In the present study an attempt has been made to find general rules common to the different crosses, which must be of interest to the plant breeder for estimating the performance of different generations and the dependence of the parents. The author first wished to include a part of this study in the above-mentioned work, but later found it more advantageous to continue the investigation for a further twelve months in order to obtain a more significant material, which is now published separately in the present study.

I. MATERIAL AND METHODS

The plant material — parents and crossings — used in this investigation is the same as was used by MANNER (1956). In the above-mentioned paper the material was extensively described. The number of crosses included is 27 (MANNER, 1956) and the number of degrees of freedom 25 in all calculations of correlation. Every variate is based on from 3 to 36 plots and on every plot 20 plants were investigated. Consequently the number of plants on which each mean (=variate) is based varies between 60 and 720 (*cf.* MANNER, 1956) grown in 3 different years. In making comparisons between parents and progeny and between different progeny generations, F_2 and F_4 were used. A sufficiency of F_1-seed could not be produced as for each plot about 250 seeds were necessary,

when the plot was 2 metres sown and the seed rate 100 germinative seeds per metre sown. Since each cross often reappeared in several trials, considerable quantities of seed would have been needed from each cross; a testing of F_1 would therefore have been very difficult to carry out. Frequent efforts were made to compare F_1 with the parents and the F_2 and F_3 generations, but the comparisons were unreliable and did not show sufficient statistical significance. This was due to two factors: on the one hand, a stand that was far too thin on account of poor germination of the seed, which in its turn led to very large plants and very high values in comparison with parents and other progeny generations; and, on the other hand, plants of the mother variety occurred in a number of cases, owing to the fact that selfing took place in some cases before castration at crossing. It therefore proved impossible to include the results of the F_1 generation, if the accepted requirements of significance were to be maintained. By first growing F_1 and then seed-harvesting only plants that were F_1 of the crosses, it was possible to avoid the danger of unintentionally using seed raised by self-pollination (MANNER, 1956).

The correlation calculations were carried out in accordance with the methods described by BONNIER and TEDIN (1940). The t^2-values were determined with the help of BONNIER and TEDIN (1940) and were denoted by asterisks: ***=P $\leqq$ 0.001 (highly significant), **=P $\leqq$ 0.01, but $>$ 0.001 (satisfactorily significant), and *=P $\leqq$ 0.05, but $>$ 0.01 (significant). Correlation coefficients with t^2-values P $\leqq$ 0.2, but $>$ 0,05, were said to have poor significance, and those with t^2-values P $>$ 0.2, to be non-significant. As a measure of the closeness of association r^2 is sometimes used in the text (FINNEY, 1953 and SPRAGUE, 1956).

Definitions

P_1 : the mother.
P_2 : » father.
Diff. $P_1 - P_2$: difference between the parents.
$\frac{P_1+P_2}{2}$: mean of the parents.
Diff. $F_2 - \frac{P_1+P_2}{2}$: difference between F_2 and the mean of the parents.
Diff. $F_2 - P_{mean}$: » » » » » » » » » .
P_{max} : the value for the parent with the higher value in the character in question.
Diff. $F_2 - P_{max}$: difference between F_2 and the parent with the higher value (the »better» parent).
Diff. $F_2 - F_4$: difference between F_2 and F_4.
r : correlation coefficient.

Investigated characters

Plant height	: the distance between the cotyledons and the uppermost capsule.
Number of flowers per plant	: this is estimated by counting the number of branch tips.
» » capsules » »	: the number of harvested and threshed capsules.
Percentage of flowers not forming capsules	: the number of flower-buds and flowers of which rests were remaining and no capsule development could be established (MANNER, 1956).
Number of seeds per plant	: the counted number of seeds per plant.
Seed weight per plant	: established by weighing, and given in milligrams.
Number of seeds per capsule	: calculated by dividing the number of seeds per plant by the number of capsules per plant.
Seed weight per capsule	: calculated by dividing the seed weight per plant by the number of capsules per plant.
Mean seed weight	: calculated by dividing the seed weight per capsule by the number of seeds per capsule.

The frequencies found are given in Fig. 1.

II. RESULTS

1. The dependence of diff. $F_2 - P_{mean}$ and diff. $F_2 - P_{max}$, respectively, on the difference between the two parents

In the material used the correlation between the difference of the parents and $F_2 - P_{mean}$ was not close. With the exception of the characters number of seeds per plant ($r = -0.0815$) and seed weight per plant ($r = -0.0259$) the correlations were positive. In the case of plant height ($r = +0.3366$), percentage of flowers not forming capsules ($r = +0.3227$), seed weight per capsule ($r = +0.2801$), the correlation coefficients showed poor significance. Positive non-significant correlation coefficients were obtained for number of flowers per plant ($r = +0.0925$), number of capsules per plant ($r = +0.0241$), and mean seed weight ($r = +0.0106$). In the correlation calculations between the differences $P_1 - P_2$ and $F_2 - P_{max}$, the positive correlation coefficient for percentage of flowers not forming capsules showed satisfactory significance ($r = +0.5676$; $t^2 = 11.8802^{**}$). The correlation coefficient for plant height showed poor significance ($r = +0.2756$). All the correlations were positive with the exception of that for number of seeds per plant ($r = -0.1806$). Positive non-significant correlation coefficients were obtained for number of flowers per plant ($r = +0.1123$), number of capsules per plant ($r = +0.0485$), seed weight per capsule ($r = +0.2034$),

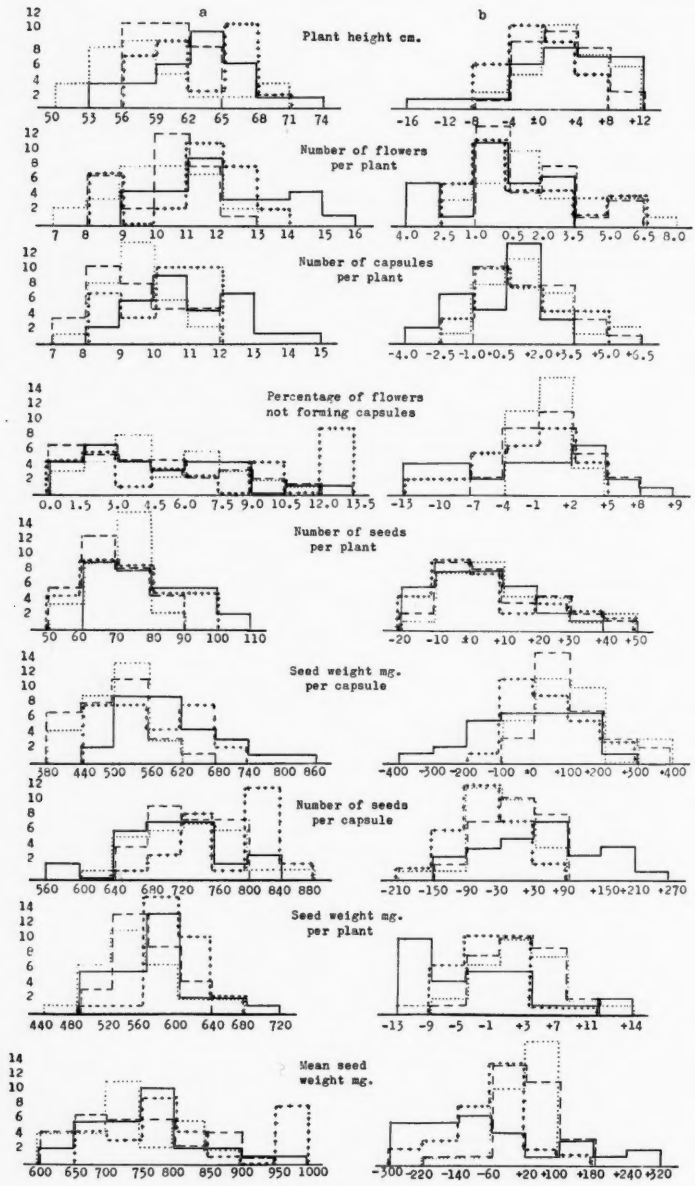
a
b
Plant height cm.
Number of flowers per plant
Number of capsules per plant
Percentage of flowers not forming capsules
Number of seeds per plant
Seed weight mg. per capsule
Number of seeds per capsule
Seed weight mg. per plant
Mean seed weight mg.

Fig. 1. Frequency diagrams.

a. – – – P_{mean} +++ P_{max} ——— F_2 ····· F_4

b. ——— P_1–P_2 – – – F_2–P_{mean} – – – F_2–P_{max} ····· F_2–F_4

number of seeds per capsule (r=+0.0552), seed weight per plant (r=+0.1402), and mean seed weight (r=+0.1978).

2. The dependence of diff. $F_2 - P_{mean}$ and diff. $F_2 - P_{max}$, respectively, on the performance of the parents

The correlations between the performance level of the parents and their progeny is calculated in different ways, namely,

1: as the correlation between P_{max} and the difference between F_2 and the mean of the parents (Tab. 1), and

2: as the correlation between the mean of the parents and the difference between F_2 and P_{max} (Tab. 2).

Correlation coefficients calculated between P_{max} and the difference between F_2 and the mean of the parents were highly significant for number of flowers per plant and showed satisfactory significance for number of seeds per plant. Statistically significant too were the correlation coefficient for number of capsules per plant, percentage of

TABLE 1. *Correlation between* P_{max} *and diff.* $F_2 - \frac{P_1 + P_2}{2}$.

Character	r	t^2
Plant height	—0.0253	0.0161
No. of flowers per plant	—0.6760	21.0287***
» » capsules » »	—0.4380	5.9327*
Percentage of flowers not forming capsules	—0.4869	7.7683*
No. of seeds per plant	—0.5100	8.7868**
Seed weight per capsule	—0.0873	0.1923
No. of seeds » »	+0.0947	0.2263
Seed weight per plant	—0.4387	5.9595*
Mean seed weight	—0.2966	2.4121

TABLE 2. *Correlation between* $\frac{P_1 + P_2}{2}$ *and diff.* $F_2 - P_{max}$.

Character	r	t^2
Plant height	—0.5263	9.5764**
No. of flowers per plant	—0.5370	10.1344**
» » capsules » »	—0.3500	3.4917
Percentage of flowers not forming capsules	—0.6581	19.1005***
No. of seeds per plant	—0.5899	14.4042***
Seed weight per capsule	—0.0137	0.0048
No. of seeds » »	—0.1084	0.2975
Seed weight per plant	—0.5009	8.3775**
Mean seed weight	—0.4560	6.5605*

flowers not forming capsules and seed weight per plant (P=0.05). The significance of the coefficient for mean seed weight was poor. In the case of plant height, seed weight per capsule and number of seeds per capsule, the coefficients of correlation were non-significant (Tab. 1). All the characters with the exception of the last-mentioned showed negative correlation.

When calculating the correlation between the mean of the parents and the difference between F_2 and P_{max}, both the characters percentage of flowers not forming capsules and number of seeds per plant showed highly significant correlation coefficients and the characters plant height, number of flowers per plant and seed weight per plant showed satisfactorily significant correlation coefficients. The correlation coefficient for mean seed weight was significant at P=0.05. Poor was the significance of the correlation coefficient for number of capsules per plant. The correlation coefficients for seed weight per capsule and number of seeds per capsule were non-significant. All the correlations were negative (Tab. 2).

The correlation coefficients for the characters, number of flowers per plant and number of seeds per plant were satisfactorily significant, calculated in both the ways used. Statistically significant in both ways calculated were the correlation coefficients for percentage of flowers not forming capsules and seed weight per plant too.

In all the cases where the correlation coefficients showed statistical significance the correlation was negative. It can thus be said that the better the performance level of the parents as regards most of the characters investigated, the smaller the difference between F_2 generation and the parents.

3. The dependence of diff. F_2—F_1 on the difference between the parents

The negative correlation coefficient for percentage of flowers not forming capsules showed poor significance (r=— 0.2967). For none of the other investigated characters did the correlation coefficients show significance. Correlation coefficients for number of flowers per plant (r=+0.0748), number of capsules per plant (r=+0.1593) and number of seeds per capsule (r=+0.0796) were positive. Negative and non-significant were the correlation coefficients for the characters plant height (r=— 0.0084), number of seeds per plant (r=— 0.0693), seed weight per capsule (r=— 0.0037), seed weight per plant (r=— 0.0122), and mean seed weight (r=— 0.2515).

4. The dependence of diff. F_2—F_4 on the performance of the parents

Correlation was calculated for the characters investigated between the performance level of the parents and the diff. F_2—F_4. As a measure of the level of performance is partly taken the mean of the parents, and partly P_{max}.

The correlation coefficient between mean of the parents and diff. F_2—F_4 was negative for number of flowers per plant ($r = -0.0773$), percentage of flowers not forming capsules ($r = -0.1133$), number of seeds per plant ($r = -0.0895$) and seed weight per plant. In the other characters with the exception of plant height ($r = \pm 0.0000$) the correlations were positive. The correlation coefficient for number of seeds per capsule showed poor significance ($r = +0.2876$). The correlation coefficients for the characters number of capsules per plant ($r = +0.1579$), seed weight per capsule ($r = +0.0649$), and mean seed weight ($= +0.0524$) were non-significant and positive.

Calculated with P_{max} as a measure of the performance level of the parents the characters, number of flowers per plant ($r = -0.1452$), percentage of flowers not forming capsules ($r = -0.0223$) and number of seeds per plant ($r = -0.1093$) showed negative correlations and all the other characters positive correlations. The significance of the positive correlation coefficient for mean seed weight ($r = +0.3385$) was poor. The other positive correlations were non-significant, namely, plant height ($r = +0.0266$), number of capsules per plant ($r = +0.1668$), seed weight per capsule ($r = +0.1243$), number of seeds per capsule ($r = +0.1604$), and seed weight per plant ($r = +0.0798$).

5. The mutual dependence of diff. F_2—F_4 and diff. F_2—P_{mean}

In Figure 2 the differences are given partly between F_2 and P_{mean} and partly between F_2 and F_4. Here the crosses are grouped after their performance in F_2. From this figure it appears that a clear dependence between diff. F_2—F_4 and diff. F_2—P_{mean} exists in the case of the characters number of flowers per plant, number of capsules per plant, number of seeds per plant, seed weight per capsule and seed weight per plant. In the case of percentage of flowers not forming capsules, number of seeds per capsule and mean seed weight the dependence seems to be smaller and the mutual dependence of the above-mentioned differences for the character plant height seems to be very small.

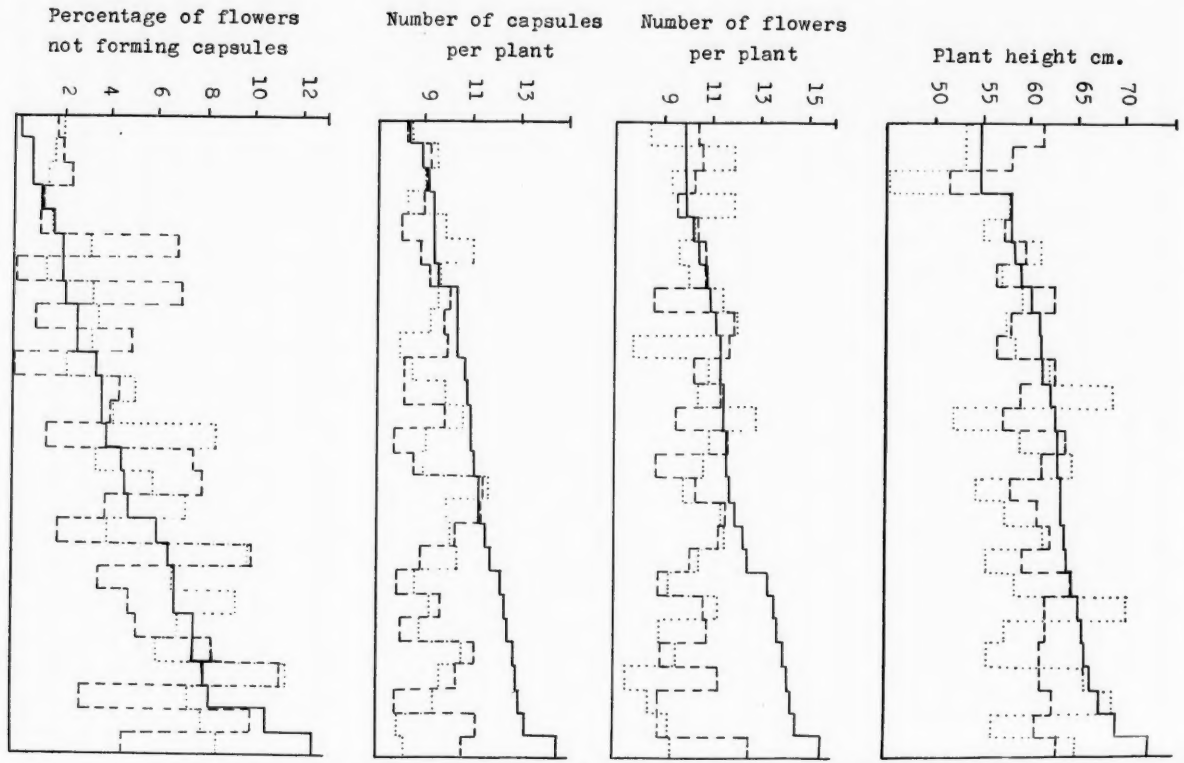

Fig. 2 a. The performance of P_{mean}, F_2 and F_4 in the studied characters for the 27 crosses.
– – – P_{mean} ——— F_2 ····· F_4

Fig. 2 a. The performance of P_{mean}, F_2 and F_4 in the studied characters for the 27 crosses.
– – – P_{mean} ——— F_2 ····· F_4

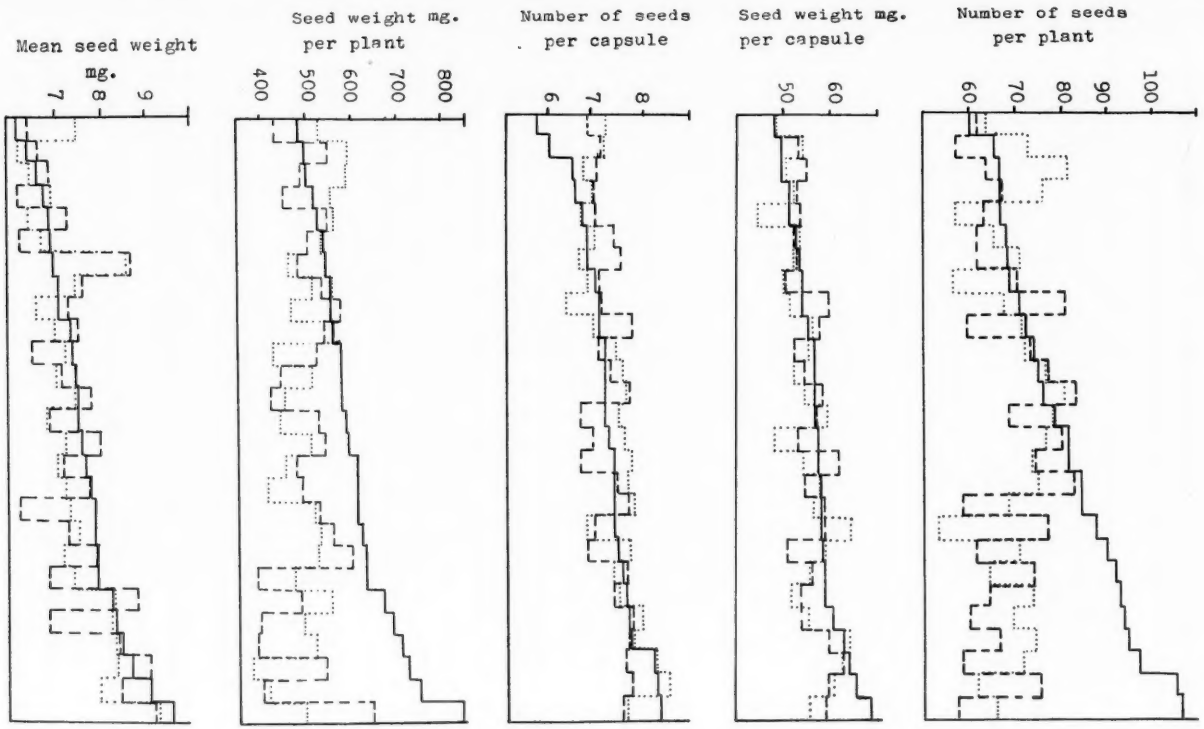

Fig. 2 b. The performance of P_{mean}, F_2 and F_4 in the studied characters for the 27 crosses.
– – – P_{mean} ——— F_2 ····· F_4

6. The dependence of the performance of the progeny on the difference between the two parents

Correlation studies were made for the nine characters investigated partly with F_2 as measure of the performance of the progeny and partly with F_4 as measure of the performance of the progeny. The correlation coefficients were positive with the exception of those for the characters number of seeds per plant ($r = -0.0753$) and mean seed weight ($r = -0.1374$), which showed negative correlation, when calculated with F_2 as measure of the performance level of the progeny. The positive correlation coefficients for the characters, plant height ($r = +0.3365$), number of capsules per plant ($r = +0.3477$), seed weight per capsule ($r = +0.3370$) and number of seeds per capsule ($r = +0.3440$) had poor significance. Positive and non-significant correlation coefficients were obtained for the characters number of flowers per plant ($r = +0.2452$), percentage of flowers not forming capsules ($r = +0.0499$) and seed weight per plant ($r = +0.1693$).

All the characters with the exception of number of seeds per plant showed positive correlation when F_4 was taken as measure of the performance level of the progeny. The character number of seeds per plant gave a non-significant negative correlation coefficient ($r = -0.0321$). Only the positive correlation coefficient for the seed weight per capsule showed significance at $P = 0.05$ ($r = +0.3837$, $t^2 = 4.3147^*$). For the other characters the significance was poor — number of capsules per plant ($r = +0.2867$), number of seeds per capsule ($r = +0.3443$) and seed weight per plant ($r = +0.2546$) — or the correlation coefficients were non-significant, namely, plant height ($r = +0.1608$), number of flowers per plant ($r = +0.2299$), percentage of flowers not forming capsules ($r = +0.2151$), and mean seed weight ($r = +0.0703$).

For the majority of the characters investigated the correlation coefficients were positive and showed poor or non-significance.

7. The dependence of the performance of the progeny on the differences $F_2 - P_{mean}$ and $F_2 - P_{max}$, respectively

This dependence, as regards the investigated characters, was studied in the following calculations:

1. the correlation between the difference of F_2 and the mean of the parents and the value for F_4; and
2. the correlation between the difference between F_2 and P_{max} and the value for F_4.

The purpose here was to see whether and to what extent the performance level of the progeny depends on the difference in performance level between F_2 and the parents.

The correlations for plant height ($r=+0.2521$), percentage of flowers not forming capsules ($r=+0.1509$), seed weight per capsule ($r=+0.2844$) and number of seeds per capsule ($r=+0.5113$) were positive. The correlations for the other five characters investigated were negative, namely, number of flowers per plant ($r=-0.2958$), number of capsules per plant ($r=-0.2200$), number of seeds per plant ($r=-0.1962$), seed weight per plant ($r=-0.4179$), and mean seed weight ($r=-0.0013$). Only as regards the number of seeds per capsule did the correlation coefficient show satisfactory positive significance ($t^2=8.8396^{**}$). Significant at $P=0.05$ was the negative correlation coefficient for seed weight per plant ($t^2=5.2909^{*}$). Negative correlation coefficient was obtained for the number of flowers per plant and positive correlation coefficient for the seed weight per capsule with poor statistical significance.

The correlation coefficients as regards the difference F_2-P_{max} and the performance of F_4 were non-significant or showed only poor significance. The correlation coefficients for the characters number of flowers per plant ($r=-0.2751$), seed weight per capsule ($r=+0.3596$) and seed weight per plant ($r=-0.3486$) showed poor significance. The correlation coefficients for the characters plant height ($r=+0.1044$), percentage of flowers not forming capsules ($r=+0.0238$), seed weight per capsule ($r=+0.3596$) and number of seeds per capsule ($r=+0.2377$) were positive and the others were negative, namely, number of flowers per plant ($r=-0.2751$), number of capsules per plant ($r=-0.1453$), number of seeds per plant ($r=-0.2498$), seed weight per plant ($r=-0.3486$) and mean seed weight ($r=-0.0005$).

8. The dependence of the performance of the progeny on the performance of the parents

The association between the performance of the progeny and the performance of the parents was studied in the following correlation studies for all the characters investigated:

1. the correlation between the performance of the mean of the parents and the performance of F_2;
2. the correlation between the performance of the mean of the parents and the performance of F_4;

3. the correlation between the performance of P_{max} and the performance of F_2, and
4. the correlation between the performance of P_{max} and the performance of F_4.

In Table 3 the coefficients are given for the correlations between the mean of the parents and the F_2. This table shows that the correlations were positive with the exception of those for the characters number of flowers per plant and number of seeds per plant. These characters' negative correlation coefficients were non-significant. The positive correlation coefficients for seed weight per capsule and mean seed weight were highly significant. Satisfactory significance was also shown by the correlation coefficients for the characters percentage of flowers not forming capsules and number of seeds per capsule. The positive correlation coefficient for plant height was significant. Poor significance was shown by the correlation coefficient for number of capsules per plant.

TABLE 3. *Correlation between the performance of* $\frac{P_1 + P_2}{2}$ *and the performance of* F_2.

Character	r	t^2
Plant height	+ 0.4380	5.9375*
No. of flowers per plant	— 0.0046	0.0009
» » capsules » »	+ 0.3542	3.5897
Percentage of flowers not forming capsules	+ 0.5083	8.7234**
No. of seeds per plant	— 0.0178	0.0795
Seed weight per capsule	+ 0.6324	16.6739***
No. of seeds » »	+ 0.5723	12.1888**
Seed weight per plant	+ 0.0425	0.0448
Mean seed weight	+ 0.6388	17.2474***

The correlations between the performance of the mean of the parents and the performance of F_4 are given in Table 4. All the correlations were positive. Highly significant was the correlation coefficient for mean seed weight. The correlation coefficients for percentage of flowers not forming capsules and seed weight per capsule showed satisfactory significance. The correlation coefficients for the characters number of flowers per plant, number of capsules per plant, number of seeds per plant, and seed weight per plant were non-significant and the coefficients for plant height and number of seeds per capsule showed poor significance.

TABLE 4. *Correlation between the performance of* $\frac{P_1 + P_2}{2}$ *and the performance of* F_4.

Character	r	t^2
Plant height	+ 0.3038	2.5424
No. of flowers per plant	+ 0.0311	0.0245
» » capsules » »	+ 0.2263	1.3487
Percentage of flowers not forming capsules	+ 0.5899	13.3435**
No. of seeds per plant	+ 0.1210	0.3712
Seed weight per capsule	+ 0.5971	13.8524**
No. of seeds » »	+ 0.3205	2.8634
Seed weight per plant	+ 0.1404	0.5033
Mean seed weight	+ 0.6850	22.1081***

In Table 5 a list is given of the correlation coefficients between P_{max} and F_2 for the investigated characters. Highly significant positive correlation coefficient was observed for mean seed weight and satisfactorily significant were the positive correlation coefficients for plant height, seed weight per capsule and number of seeds per capsule. The positive correlation coefficient for percentage of flowers not forming capsules was also statistically significant. The positive correlation coefficient for number of capsules per plant showed poor significance. The negative correlation coefficients for number of flowers per plant and number of seeds per plant were non-significant and also the positive correlation coefficient for seed weight per plant.

TABLE 5. *Correlation between the performance of* P_{max} *and the performance of* F_2.

Character	r	t^2
Plant height	+ 0.5506	10.8765**
No. of flowers per plant	— 0.2100	1.1537
» » capsules » »	+ 0.2748	2.0413
Percentage of flowers not forming capsules	+ 0.4772	7.3739*
No. of seeds per plant	— 0.0067	0.0009
Seed weight per capsule	+ 0.5504	10.8577**
No. of seeds » »	+ 0.5552	11.1359**
Seed weight per plant	+ 0.0811	0.1657
Mean seed weight	+ 0.6040	14.3495***

In Table 6 the coefficients are given for the correlations between the performance of P_{max} and the performance of F_4. The correlations were

TABLE 6. *Correlation between the performance of P_{max} and the performance of F_4.*

Character	r	t^2
Plant height	+ 0.3067	2.5964
No. of flowers per plant	— 0.0044	0.0005
» » capsules » »	+ 0.1135	0.3259
Percentage of flowers not forming capsules	+ 0.5057	8.6190**
No. of seeds per plant	+ 0.1821	0.8576
Seed weight per capsule	+ 0.4537	6.4804*
No. of seeds » »	+ 0.4446	6.1609*
Seed weight per plant	— 0.0071	0.0013
Mean seed weight	+ 0.4545	6.5077*

positive with the exception of those for the characters number of flowers per plant and seed weight per plant. Satisfactory significance was shown by the positive correlation coefficient for the percentage of flowers not forming capsules. Statistically significant were the positive correlation coefficients for seed weight per capsule, number of seeds per capsule and mean seed weight too. The significance for the correlation coefficient of plant height was poor. The coefficients for both the abovementioned negative correlations and the positive correlations for number of capsules per plant and number of seeds per plant were non-significant.

III. DISCUSSION

In the material used the correlations between differences $P_1 - P_2$ and $F_2 - P_{mean}$ and $F_2 - P_{max}$, respectively, were not close. Significant correlation coefficients could have been expected seeing that several investigators have found association between heterosis and the relationship between the parents. Thus, WEXELSEN (1945) found in red clover a high degree of heterosis when crossing unrelated inbred families that showed »depressed vigor». SPRAGUE (1946) considered that »in general the highest yielding double crosses are those that combine single crosses differing most widely in parentage». He added later that it is indicated by available data that »dominant favourable genes are more important than physiological stimulation resulting from genetic diversity». COWAN (1943) found highly significant positive correlation between »top cross yields of unrelated inbred and their yields in single crosses and predicted double crosses». COWAN found no correlation when the inbred used as parents were related. EAST and HAYES (1912) and EAST (1936)

were of the opinion that the heterosis is dependent on the genetic disparity and increases on an average with increased genetic disparity between the parents. JOHNSON and HAYES (1940) concluded that »diversity in genetic origin is an important factor in obtaining the maximum expression of hybrid vigor», and HAGBERG (1952 b), in his investigation on rye, considered that he had found »an indication that the degree of heterosis is parallel to the degree of genetical differentiation between the populations».

In conformity with VEATCH (1930), HARRINGTON (1940), IMMER (1941), and CARNAHAN (1947) the present author has compared F_2 with the parents. This difference is not as large as the difference between the performance for F_1 and the performance for parents, when heterosis is found (CARNAHAN, 1947; MANNER, 1956), but most however be considered to be of importance for estimating the performance of the progeny in comparison with the performance of the parents.

It can be established that the correlations between the differences in performance between the parents and diff. $F_2 - P_{mean}$, respectively, were weak or non-existent, and that the correlation coefficients showed poor significance or were non-significant with one exception only. That the correlations were so weak in comparison with the correlations found by other workers between the »genetic disparity» between the parents and heterosis can be interpreted as depending on the fact that the difference between P_1 and P_2 is no measure of the relationship between the parents used, which naturally is an objection which the present author has kept in view. But it cannot be asserted that it may be due to the material used here being other than that used by other investigators especially when concrete exceptions from their conclusions have been previously given regarding the same *Linum*-material as is used as basis for the present study (MANNER, 1956).

From time to time reference has been made in different papers to the fact that a negative correlation between the parental performance level and difference between parental and progeny generations is predominant. In the present study these opinions have also been shown to be valid for the flax material used here. The correlation coefficients showed significance in most of the calculations, but the highest r^2 was 0.4570, which entails that 46 per cent of the total variation in difference between F_2 and the parents was dependent on the performance level of the parental generation. In most cases this percentage is much smaller. Only in a few cases is this percentage larger than 30. The percentages are as follows:

Character	$\frac{P_{max}}{F_2 - \frac{P_1+P_2}{2}}$	$\frac{\frac{P_1+P_2}{2}}{F_2 - P_{max}}$
Plant height	0	28
Number of flowers per plant	46	29
» » capsules » »	19	12
Percentage of flowers not forming capsules	24	43
Number of seeds per plant	26	35
Seed weight per capsule	1	0
Number of seeds per capsule	0	1
Seed weight per plant	19	25
Mean seed weight	9	21

The diff. F_2—F_4, inbreeding depression between both these generations showed no large dependence on the difference in performance level between the parents. In not so much as one single case was 10 per cent of the variation in the difference between the parents associated with the variation in inbreeding depression. In most cases this percentage was much smaller. In the correlation calculation between the performance level of parental generation and the diff. F_2—F_4 the coefficients were small.

The dependence of diff. F_2—F_4 on the diff. F_2—P_{mean} seems to be considerable as regards all characters except plant height. The material from these crosses that have shown a high diff. F_2—P_{mean} and diff. F_2—F_4 should give a progeny showing only poor heterosis in general, if the opinion of KIESSELBACH (1922) is correct. He wrote, namely, that »the general concensus of opinion among students of this problem would seem to support the theory that those elemental strains which undergo the least reduction from inbreeding are likely to produce the most productive hybrid offspring when crossed». The results of KIESSELBACH's own investigations in maize generally confirmed this; he wrote »there appears to be some general correlation between productivity of the pure line parents and that of their hybrid offspring. Exceptions to this general relation occur». This must be understood to imply that this is valid when using one of the first generations — in the first place F_1 — and that when a later generation is used the combining ability is specific for different genotypes. On the basis of the results of the present study it is not possible to say whether the possibilities of finding frequently a large number of individuals with good combining ability in populations, which in the progeny of the crosses show good combining ability and then show a high degree of inbreeding depression, are smaller than when taking individuals from populations showing none or only a small

degree of heterosis and inbreeding depression. In the present study the association between the diff. $F_2 - P_{mean}$ and the diff. $F_2 - F_4$ seems to be very clear (Fig. 2) for the characters number of flowers per plant, number of capsules per plant, number of seeds per plant, seed weight per capsule and seed weight per plant, but much smaller for the character plant height.

No large part of the total variation in the difference between the parents was found in progeny. The dependence for F_2 was at highest 12 per cent and the dependence for the seed weight per capsule in F_4 was nearly 15 per cent. A closer corrrelation might have been expected between the difference in performance level of the parents and F_2 than for F_4, having regard to the rather close correlation in some cases between diff. $F_2 - P_{mean}$ and $F_2 - F_4$, but no such clear tendency could be found.

The correlation between the diff. $F_2 - P_{mean}$ and diff. $F_2 - P_{max}$, respectively, and the performance of F_4 was calculated. The highest percentage of the variation in F_4 that is associated with the variation in difference between parental and progeny generations is about 26 per cent, namely, for number of seeds per capsule.

The coefficients for the correlations between the performance of the parental generation and the performance of the progeny (Tabs. 3, 4, 5 and 6) were in most cases positive and showed in many cases significance. The characters whose correlation coefficients were non-significant or showed poor significance in all calculations were number of flowers per plant, number of capsules per plant, number of seeds per plant and seed weight per plant. The others, plant height, percentage of flowers not forming capsules, seed weight per capsule, number of seeds per capsule and mean seed weight had significant correlation coefficients. Many investigators have studied the correlation between different generations in different plants — both self-pollinated and cross-pollinated. »A good relationship between the performance of parents and the average performance of the crosses» was found by CARNAHAN (1947) in flax. MANNER (1956), in his investigation, gives an example of an exception. In the present investigation in flax it appears that the correlation in some cases was not close. Exceptions have also been found by other investigators in other plant species. BURDICK (1954), for instance, found »notable exceptions» from the average relationship in earliness in tomato between parents and their hybrids. HARRINGTON (1940) considered that bulk F_2-tests can be used as indication of the yielding potentialities of wheat crosses, and he stated that bulk F_2-tests may have a supple-

mentary indicative value as to the yielding potentialities. Highly significant correlation coefficients were found by LARSON (1941) between the parental mean and the F_1 hybrid in tomato. He studied the following characters: total yield, earliness, fruit size and fruit shape.

In maize many investigators have studied the dependence of the progeny on the parents. A brief review of the results are given below. JORGENSON and BREWBAKER (1927) found positive significant correlations between the mean of the parental lines and F_1 in the following characters: yield in grams per hill, length of ear, diameter of ear, number of kernel rows per ear, height of stalk and weight of seed. In maize NILSSON-LEISSNER (1927) found significant positive correlations between the parental lines and the F_1 generation in yield, length of ear, diameter of ear, number of kernel rows, percentage of second ears and height of plant. He summarized »crosses between the more vigorous selfed lines yield better, on the average, than crosses between less vigorous lines». In investigations in maize JENKINS (1929) studied inter alia the correlations between characters in the inbred parental lines and the same characters in their crosses. JENKINS calculated the correlation coefficient for yield and 18 other characters (date of tasseling, date of silking, shrinkage of the harvested ears, plant height, nodes per plant, nodes below ear, percentage of nodes below ear, plants smutted, plants erect at harvest, ears moldy, suckers per 100 plants, plants with two or more ears, ears per plant, ear length, ear diameter, ear-shape index, kernel rows per ear and shelling percentage). The investigator calculated both with each parent separately and with the parental means. The highest coefficients of correlation were — calculated after both methods found for plants erect at harvest ($r=0.51$ and $r=0.72$) and kernel rows per ear ($r=0.47$ and $r=0.67$). The lowest correlation coefficients were found for yield ($r=0.14$ and $r=0.20$). As regards all characters the correlation coefficients were positive. O'KELLY and HULL (1932) obtained in maize, when comparing parent and progeny in the characters grain percentage and yield, positive correlations in most cases but these correlation coefficients were »of doubtful significance»; and JENKINS and BRUNSON (1932) calculated the coefficients of correlation in maize for the characters: date $^1/_4$ tasseled, date $^1/_4$ silked, date of maturity, moisture at harvest per cent, plants with burned leaves per cent, plants with burned tassels per cent, plant height, ear height, plants erect per cent, plants smutted per cent, stalks per plant, ears per plant, ears per hundredweight, ear length, shelling per cent, test weight per bushel and acre yield, between »the mean performance of the single crosses of inbred lines» and »the per-

formance of these lines in crosses with a commercial variety»; most of the correlation coefficients were significant. DAVIS (1934) studied in maize the correlation between parents and progeny and found positive correlations.

Several investigators have studied the forage grasses and found parent-progeny correlation. Of these MYERS and CHILTON (1941) observed close correlation between »the mean winter injury of the parental clones and their inbred progenies» both in orchard grass and timothy. WEISS, TAYLOR and JOHNSON (1951) investigated orchard grass and found significant correlation coefficients between the parental clones and their open-pollination progenies for the characters leaf width, panicle number and winter survival. Between the mean of the parents and the performance of single crosses they found correlations for winter survival, leafiness, leaf width, panicle number, leaf diseases and lodging. Significant correlation coefficients between parental plants and their inbred progenies in several characters in orchard grass were found by KALTON, SMIT and LEFFEL (1952). The correlation coefficients ranged between +0.52 (second-cutting yields) to +0.79 (panicle number). Both yield and plant height showed significant correlation coefficients between parents and progenies in orchard grass (OLDEMEYER and HANSON, 1955). Several investigators have studied the relationship of open-pollination progeny generations to their parents. In controlled crosses KNOWLES (1950) obtained positive correlation for forage yield between the open-pollination progeny and the parental clones. The coefficients of correlation were non-significant. Whereas MCDONALD, KALTON and WEISS (1952) and HAWK and WILSIE (1952) found significant positive correlation coefficients in the corresponding correlation, but the correlation coefficient obtained by MCDONALD, KALTON and WEISS (1952) for the association between the first inbred generation and their parental clones was non-significant. Association between parental clones and their polycross progenies was found by HITTLE (1954) in smooth bromegrass. Between parent and progeny RAEBER and KALTON (1956) found relationship in *Bromus inermis*.

Results have thus been obtained especially in maize and forage grasses, but in other plants species too that show correlation between the performance of the parents and the progeny. These results agree with the present study. Other investigators have also given information on association for characters in inbred parents in relation to their progeny (KIESSELBACH, 1922; RICHEY, 1924; LINDSTROM, 1931; HAYES and JOHNSON, 1939; POWERS, 1945; KELLER, 1949 and others). Many invest-

igators have studied and discussed the possibilities of predicting the performance of the progeny by the performance of the parents.

That the performance of double-cross corn hybrids can be predicted with accuracy from the average performances of the recombination single crosses, has been shown by several investigators (JENKINS, 1934; DOXTATOR and JOHNSON, 1936; ANDERSON, 1938; HAYES, MURPHY and RINKE, 1943; HAYES, RINKE and TSIANG, 1946 and PETERSEN, ELDREDGE and JOHNSON, 1949). SPRAGUE (1946) stated that in maize single-cross performance data can be used for prediction of the performance of double-crosses. The best method for estimation »is based on the average performance of the four non-parental single-cross combinations». HAYES, MURPHY and RINKE (1943) concluded that the »differences in yield and moisture content between predicted and actual 'performances' were not so large that they would greatly affect the relative performance of hybrids». On the basis of the present study it must be stated that the possibilities of predicting in flax the performance of the progeny generations, F_2 and F_4, on the basis of the parents, seem to be rather small, but that the possibilities appear to be different for different characters.

The generally positive correlation coefficients for the different characters between the performance of the parental generation and F_2 showed significance, but the correlation cannot be said to be close. When using parental mean in the correlation the closest correlation was for mean seed weight and for seed weight per capsule, for which characters about 40 per cent of the total variation in F_2 depended on the parental generation. For the other characters the percentages were smaller, which was the case when P_{max} was used as measure of the performance level of the parental generation too. The percentages of variation in F_2 that depended on the parental generation in the present calculations are given below:

Character	$\frac{P_1 + P_2}{2}$ / F_2	P_{max} / F_2
Plant height	19	30
Number of flowers per plant	0	4
» » capsules » »	13	8
Percentage of flowers not forming capsules	26	23
Number of seeds per plant	0	0
Seed weight per capsule	40	30
Number of seeds per capsule	33	31
Seed weight per plant	0	1
Mean seed weight	41	36

The positive correlations between performance level of the parental generation and the performance of F_4 were only in some cases relatively close. The largest r^2-values were found when the parental mean was taken as measure of the parental generation. For the characters mean seed weight, seed weight per capsule and percentage of flowers not forming capsules the r^2-values were 0.4692, 0.3565 and 0.3480, respectively. These r^2-values show that about 47, 36 and 35 per cent of the variation in parental generation was found in F_4. The percentages of association are given below:

Character	$\frac{P_1+P_2}{2}$ / F_4	P_{max} / F_4
Plant height	9	9
Number of flowers per plant	10	0
» » capsules » »	5	1
Percentage of flowers not forming capsules	35	26
Number of seeds per plant	1	3
Seed weight per capsule	36	21
Number of seeds per capsule	10	20
Seed weight per plant	2	0
Mean seed weight	47	21

WRIGHT (1935) analysed the variance in 1. additive gene effects, 2. dominance deviations, 3. epistatic deviations, 4. environmental effects and 5. non-additive joint effects of heredity and environment. Further discussions regarding epistasis have been published by SENTZ, ROBINSON and COMSTOCK (1954), and JINKS (1955). GRAFIUS, NELSON and DIRKS (1952) divided the total genetic variance into additive genetic variance (heritable genetic variance) and a remainder into dominance deviations and epistasis. They wrote »a genetic interpretation of the variance involved in early generation yield testing of bulked progenies of different barley crosses was presented. The non-heritable fraction comprised the major proportion of the genetic variance in the F_2. In the F_3, with increased homozygosity, the non-heritable fraction decreased in comparison to the additive (heritable) fraction of the genetic variance». This argumentation agrees very well with the results of the present study. Thus, the differences between r^2-values for correlation coefficients between $\frac{P_1+P_2}{2}$ and F_2, and between $\frac{P_1+P_2}{2}$ and F_4, respectively, were on the whole relatively small, but the differences between the r^2-values for the correlations between P_{max} and F_2 were considerably larger than

the r^2-values for the correlations between P_{max} and F_4. The last-mentioned correlation naturally gave the smaller r^2-values.

In this connection it is of interest to discuss the value of parent-progeny correlation as a measure of to what extent it is possible to predict the performance of the progeny on the basis of the performance of the parents and to use the prediction in practical plant breeding. THOMAS and KERNKAMP (1954) considered that heritability ratios are »more convenient and useful as an aid to selection» than the correlations between the parental generation and the progeny.

The difference between $r^2 \times 100$ and heritability % has been pointed out by THOMAS and KERNKAMP (1954) who state that »the meaning of heritability may be thought of as r^2 if we could correlate genetic variability of clones (x) with phenotypic variability of their progenies (y). Of course we are not able to measure the genotypes directly and therefore cannot compute r directly».

Heritability or the ratio of additively genetic variance to the total variance has been studied in several plant species. Several authors have estimated the genotypic and environmental portions of the variance in populations or the genotypic and phenotypic correlations (WEBER and MOORTHY, 1952; ROBINSON, COMSTOCK and HARVEY, 1951; MAHMUD and KRAMER, 1951; BURTON, 1951; KALTON, SMIT and LEFFEL, 1952; RAEBER and KALTON, 1956). Many investigators have estimated the heritability (MCDONALD, KALTON and WEISS, 1952; HITTLE, 1954; WARNER, 1952; BARTLEY and WEBER, 1952; RAEBER and KALTON, 1956; HANSON, ROBINSON and COMSTOCK, 1956).

When comparing parents and progeny with each other many investigators have used the performance of the mean of the parents as measure of the parents (KIESSELBACH, 1922; VEATCH, 1930; HARRINGTON, 1940; IMMER, 1941; ABBE and WANG, 1943; GRANHALL, 1946; PLONKA, 1951; GARTNER, HANEY and HAMNER, 1953; TURNER, 1953 and MANNER, 1956). VEATCH (1930), and partly MANNER (1956), and others, have parallelly compared the progeny with the parent having the higher values. Other investigators, for example, WHALEY (1944) and HAGBERG (1952 a), have been of the opinion that it is more correct to use the »better» parent for such comparisons. In the present investigation, both the mean of the parents and the parent with the higher value are in many cases parallelly used as measure of the performance of the parents, in the cases where the same numerical material is not used for calculation of both x- and y-variates, *i. e.*, when the x- and y-variates are represented by independent observations as inter alia x=a and

y=b, whereas the correlations calculated on the basis of x=a, y=a—b and x=a—b, y=a—c are *avoided*, because x and y are in such cases calculated on the basis of numbers which are partly the same. In some cases, however, the calculation is made on the basis of variates of the type $x=a$, $y=b-\frac{a+c}{2}$ (Tab. 1). The present author knows very well that in such correlation calculations y is partly calculated on *a* too, and that this causes an error which, however, the present author considers to be small.

In calculating correlations with the parental generation most investigators have used the mean of the parents (LARSON, 1941; JENKINS, 1929; JORGENSON and BREWBAKER, 1927; VEATCH, 1930; WEISS, TAYLOR and JOHNSON, 1951), others have however used the »better» parent or both parents separately (JENKINS, 1929; VEATCH, 1930). As to the possibilities of obtaining a correlation between the higher yielding parent and F_1 LARSON (1941) wrote: »it is evident that the factors for greater yield are dominant in most F_1 hybrids, but the complementary effect of genes for yield, in several crosses, produces an F_1 that is higher yielding than either parent. It is therefore impossible to obtain a significant correlation between the dominant higher yielding parent and the F_1 since there is no uniform change of one variable with the other».

Acknowledgements. — My most sincere thanks are due to miss BRIGITTE BRETSCHNEIDER for valuable help in carrying out this study.

SUMMARY

(1) For most of the investigated characters a positive correlation was found between the difference in performance for the parents used and diff. F_2—P_{mean} and P_{max}. The correlation coefficients were non-significant or showed poor significance with the exception of those for percentage of flowers not forming capsules, which coefficient showed satisfactory significance when P_{max} was used as measure of the performance of the parental generation. Estimated in conformity with both calculation methods — using the mean of the parents and P_{max}, respectively, as measure of the performance of the parents — the correlation coefficient for number of seeds per plant was negative, but non-significant.

(2) Estimated in two different ways all the correlation coefficients — with one exception — were negative between the performance of the

parental generation and diff. $F_2 - P_{mean}$, and diff. $F_2 - P_{max}$, respectively. In one or more of the calculations all characters, with the exception of seed weight per capsule and number of seeds per capsule, gave correlation coefficients showing significance (Tabs. 1 and 2).

(3) The correlation coefficients for the relationship between the difference of performance for the parents used and the diff. $F_2 - F_4$ were non-significant for all characters except for percentage of flowers not forming capsules, which correlation coefficient was negative and showed poor significance. The correlations for number of flowers per plant, number of capsules per plant and number of seeds per capsules were positive and the others negative.

(4) The correlation coefficients for the relationship of the performance level of the parents and the diff. $F_2 - F_4$ were in most cases non-significant or showed only poor significance. The correlations for number of capsules per plant, seed weight per capsule, number of seeds per capsule and mean seed weight were positive and the correlations for number of flowers per plant, percentage of flowers not forming capsules and number of seeds per plant were negative in both series.

(5) Between diff. $F_2 - F_4$ and diff. $F_2 - P_{mean}$ there seemed to exist a clear dependence in the present material as regards the characters number of flowers per plant, number of capsules per plant, number of seeds per plant, seed weight per capsule and seed weight per plant. Possibly there existed a smaller dependence in the case of the characters percentage of flowers not forming capsules and mean seed weight (Fig. 2).

(6) The correlations between the difference between the parents used and the performance level of F_2 and F_4, respectively, were in most cases positive, but the correlation coefficients were non-significant or showed poor significance with the exception of the coefficient for seed weight per capsule in the calculation for the performance level of F_4, which coefficient was significant at $P = 0.05$.

(7) Positive correlation coefficients were obtained for most characters when calculating the correlation between the performance of the parental generation and F_2. Statistical significance was shown by the correlations for the following characters: plant height, percentage of flowers not forming capsules, seed weight per capsule, number of seeds per capsule and mean seed weight. The significance for number of capsules per plant was poor and the correlations for number of flowers per plant, number of seeds per plant and seed weight per plant were non-significant (Tabs. 3 and 5).

(8) The correlations between the performance level of the parental generation and F_4 were positive except for the characters number of flowers per plant and seed weight per plant when calculated with P_{max} as measure of the performance level of the parental generation. The significance of the correlation coefficient for number of seeds per capsule was poor when the parental mean was used, and significant at $P=0.05$ when P_{max} was used as measure of the performance of the parental generation. The significance of the correlation coefficients for plant height was poor, and the coefficients for number of flowers per plant, number of capsules per plant, number of seeds per plant and seed weight per plant were non-significant (Tabs. 4 and 6). In both calculations the correlation coefficients for percentage of flowers not forming capsules, seed weight per capsule and mean seed weight were statistically significant.

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THE INCIDENCE OF CONSANGUINEOUS MARRIAGES IN SWEDEN[1]

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(Received November 28th, 1957)

IN the study of population genetics it is important to estimate the incidence of consanguineous marriages in different countries and in localities within a single country. Where the Roman Catholic Church is prevalent, estimates of the incidence of consanguineous marriages can be obtained by a reasonable amount of work, since the special »dispensa» required for such marriages is registered in the parish books. In some countries particular types of marriages between relatives enter the official statistics. This source of information has not, however, been widely used and relatively few data are on record.

In countries in which consanguineous marriages do not encounter a special registration the amount of work necessary to obtain good estimates is considerable. Where parish books are carefully kept (as they are in most instances in Sweden) the best estimates are obtained by following the genealogies of single families in these books. The student has of course to decide which families to investigate, *e. g.* the families resident in a certain area at the time of the investigation, the families with both partners born in the same area, the families of children born within a certain period of time, etc. This method is painstaking and practically confined to the study of small communities. An alternative, but less efficient, method is to estimate the rate of consanguinity in a sample of the general population, *e. g.* patients in hospitals, parents of school children, etc. (see NEEL *et alii*, 1949, for discussion).

This paper presents all the available information on the incidence of consanguineous marriages in Sweden, with particular emphasis on first cousin marriages. The State Institute for Human Genetics in Uppsala has long been interested in the effects of inbreeding on human populations, both from a theoretical point of view (WAHLUND, 1928; DAHL-

[1] A paper read before the 1st International Congress of Human Genetics, Copenhagen, August 1--6, 1956.

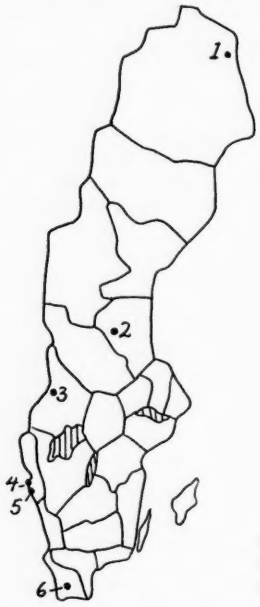

Fig. 1. Location of the rural communities in which the incidence of first cousin marriages has been estimated (*cf.* Tab. 1).

BERG, 1929, 1938, 1947) and by collection of actual data (BÖÖK, 1948, 1953, 1956, 1957; BÖÖK and MÅWE, 1955; ROMANUS, 1953). Additional relevant information has been obtained from the papers of LARSSON and SJÖGREN (1954) and LARSON (1956).

INCIDENCE OF FIRST COUSIN MARRIAGES DETERMINED BY THE GENEALOGICAL METHOD

Five surveys have been made in Sweden by the genealogical method. The results are given in Table 1, while Fig. 1 shows the geographical localization of the localities (or parishes) investigated. They are fairly representative of the different types of rural communities to be found in Sweden. Figures on the incidence of first cousin marriages should always be complemented with information on the demographic and dynamic aspects of the population concerned. A few such data will be given here as a comment to Table 1, while the original papers should be consulted for more details. The unpublished data will be commented on more extensively.

TABLE 1. *Data on the incidence of first cousin marriages, determined by genealogical method in six rural communities in Sweden.*

Number defining the area in text and Fig. 1	Locality	No. of marriages	First cousin marriages		Period in which the marriages have been contracted	Total area (sq. Km.)	Population size at (year)	Source	Remarks
			No.	Per cent					
1	Muonionalusta	191	13	6.81	1890—1946	536	1415 (1949)	Böök (1948)	
1	Junosuando	281	8	2.85	1890—1946	471	1963 (1949)	» »	
1	Pajala	843	8	0.95	1890—1946	840	5603 (1949)	» »	
2	Ovanåker	2098	12	0.57	1900—1944	520	7239 (1951)	Unpublished data	
3	Östmark	570	7	1.23	1905—1925	397	2862 (1950)	Böök and Måwe (1955)	No. of marriages is the number of existing marriages with both partners resident in Östmark
3	»	535	7	1.31	1910—1941				
4	Orust	468	11	2.35	—	403	12514 (1950)	Larsson and Sjögren (1954)	No. of marriages is the number of marriages contracted between parents of children born in 1861—1920
5	Tjörn	324	10	3.09	—	166	8687 (1950)	»	
6	Börringe	576	10	1.74	1901—1952	55	1187 (1952)	Larson (1956)	No. of marriages is the number of marriages actually contracted in Börringe

Locality 1. — The three parishes of Muonionalusta, Junosuando and Pajala are located in the North of Sweden (Norrbotten) near the Finnish border. The district is geographically relatively isolated, with a pronounced rural character. It is characterized by high birth rates and low immigration rates. The general mobility of the population, measured by crude migration rates, is low. The northernmost, and therefore the most geographically isolated of the three parishes (Muonionalusta) shows the highest incidence of first cousin marriages. An analysis of its genetical demography (BÖÖK, 1956 a) indicates that the population of the total area should not be regarded as one breeding unit but as a minimum of three. BÖÖK estimated the mean effective population size as 150, 300 and 650 in the three parishes for the period 1900—1950.

TABLE 2. *Parish of Ovanåker. Crude migration rates, 1900—1944.*

Period	Average population size	In-migration per year per thousand	Out-migration per year per thousand
1900—1909	4330	20	30
1910—1919	4910	26	25
1920—1929	5779	28	27
1930—1939	6206	25	27
1940—1944	8041	35	35

Locality 2. — The parish of Ovanåker is located in middle East Sweden (Hälsingland). It is mostly a rural community but industrial activity has developed in recent years. 80 per cent of the area is covered by forest. It has good communications and no geographical barriers. The population has increased in the last fifty years, totalling 4212 in 1900 and 7239 in 1951. This increase is due more to higher fertility than to immigration. The mobility of the population is rather high but emigration has been equalled by immigration in recent years. Crude migration rates are given per 1000 average inhabitants for different periods in Table 2. The 2098 marriages registered in the period 1900—1944 are distributed according to the birth place of the partners in Table 3, which gives another rough estimate of the mobility of the breeding population. The incidence of first cousin marriages is 0.57 per cent.

Locality 3. — The parish of Östmark is located in middle West Sweden (Värmland), near the Norwegian border. The region is geographically relatively isolated, has a rural character. The population is

TABLE 3. *Parish of Ovanåker. Distribution of the partners of the marriages investigated according to the place of birth.*

Partners born in Ovanåker	No.	Per cent
Both	901	42.9
One	1028	49.0
None	169	8.1
Total	2098	

decreasing (from 1861 to 1950) both due to emigration and to decrease in the average number of children per family. Return immigration has been high in recent years so that the general mobility of the population, judged by crude migration rates, is rather low. The incidence of first cousin marriages is practically the same when determined in 1925 (1.2 per cent) and in 1954 (1.3 per cent). The effective population size for the period 1925—1954 has been estimated by BÖÖK and MÅWE (1955) between 100 and 300.

Localities 4 and 5. — These consist of 13 parishes located in the two large islands of Orust and Tjörn and in a number of small islands of the West Coast. The population has a rural character and a relatively low mobility. The population has decreased in the last fifty years, emigration is high, but return immigration is also high. Immigration of people born outside the area is practically negligible. The marriages investigated are here those contracted between parents of children born in the period 1861—1920. The incidence of first cousin marriages given in Table 1 refers to »native» population. LARSSON and SJÖGREN (1954) give also the frequency in the »resident» population, as 2.9 and 2.0 per cent for Tjörn and Orust respectively. The weighted figures for the whole area, are 2.6 and 2.3 per cent for the two types of population.

Locality 6. — The parish of Börringe is located in South Sweden (Malmöhus), the most densely populated part of the country. It is a rural community with a moderately mobile, decreasing population. It has very good communications and no geographical barriers.

The incidence given in Table 1 and Fig. 1 is calculated on the total number of marriages contracted in the parish. LARSON (1956) gives two alternative estimates, based on a different classification of the marriages. These are 2.2 and 1.9 and do not depart significantly from the one, elaborated in Table 1.

INCIDENCE OF CONSANGUINEOUS MARRIAGES DETERMINED BY OTHER METHODS

ROMANUS (1953) determined the frequency of all types of marriages between relatives among the applicants for legal abortion in the period 1946—1950 and among their parents. All the social classes were represented in this sample, which, however, is not representative of the general population because of the particular kind of selection on which it is based. Extramarital and illegal (*e. g.* incest) relations are included. Table 4 shows the results for the whole country. ROMANUS also gives the geographical distribution of the sample. Worth mentioning here are the figures on the incidence of first cousin marriages in the towns, which are given in Table 5. The incidence of first cousin marriages is always higher among the parents of applicants.

Complete genealogies of the Swedish nobility have been published in nine volumes by ELGENSTIERNA (1925—1936). The frequency of con-

TABLE 4. *Incidence of consanguineous marriages and of parental consanguinity among the applicants for legal abortion in Sweden.*

	No.	First cousin marriages		Other consanguineous marriages		Total	
		No.	Per cent	No.	Per cent	No.	Per cent
Applicants	15802	65	0.41	78	0.49	143	0.90
Parents of applicants	15802	109	0.69	69	0.44	178	1.13

TABLE 5. *Incidence of first cousin marriages among applicants for legal abortion (first line) and their parents (second line), in towns.*

Town	No. of applicants	First cousin marriages	
		No.	Per cent
Stockholm	4723	13	0.28
		22	0.47
Göteborg and Malmö	1982	10	0.50
		15	0.76
Other towns (Visby excluded)	4766	17	0.36
		27	0.57

TABLE 6. *Incidence of consanguineous marriages in the Swedish nobility. 1821—1930.*

Period	No. of marriages	First cousin marriages		Other degrees of consanguinity	
		No.	Per cent	No.	Per cent
1821—1830	393	24	6.11	16	4.07
1851—1860	621	31	4.99	13	2.09
1871—1880	558	19	3.41	7	1.25
1901—1910	662	2	0.30	3	0.45
1921—1930	368	3	0.82	9	2.45

sanguineous marriages has been extracted from these books by the late G. DAHLBERG and not published before. Table 6 gives the frequency of first cousin marriages and of other types of consanguinity in the nobility sample from 1821 to 1930, divided into ten-year periods. The total incidence of first cousin marriages for the period considered is 3.04 per cent. It has decreased from 6.11 per cent in 1821—1830, to 0.82 per cent in 1921—1930. Table 7 gives the distribution of marriages between noble partners born in the same »kommun» (*i. e.* urban or rural district), respectively in the same »härad» (*i. e.* small jurisdictional district), or »län» (*i. e.* county). It is interesting to note that also this particular section of the population has followed the trend of a decrease in the rate of consanguineous marriages observed in the general population. The figures in Table 6 and 7 give an estimate of the rate of disappearance of this peculiar type of »isolate».

Table 8 shows the frequency of parental consanguinity in a sample of hospital patients from one hospital in Uppsala and one in Stockholm.

TABLE 7. *Distribution of the marriages of the Swedish nobility according to the birth-place of the partners.*

Period	No. of marriages	Both partners are born in the same					
		kommun		härad		län	
		No.	Per cent	No.	Per cent	No.	Per cent
1821—1830	393	25	6.36	38	9.67	99	25.19
1851—1860	621	52	8.37	63	10.14	167	26.89
1871—1880	558	39	6.99	47	8.42	123	22.04
1901—1910	662	50	7.55	67	10.12	121	18.28
1921—1930	368	27	7.34	31	8.42	71	19.29

TABLE 8. *Incidence of parental consanguinity in patients from two hospitals.*

Hospital	No. of patients	Patients with parental consanguinity	
		No.	Per cent
Akademiska sjukh., Uppsala	5284	26	0.49
S:t Eriks sjukh., Stockholm	1807	18	1.00
Total	7091	44	0.62

The overall incidence of consanguineous parents is 0.62 per cent. The difference between the two hospitals (0.51 ± 0.25) is high but is likely to be due to chance.

DISCUSSION

The first conclusion which may be extracted from the observed incidence of first cousin marriages in the Swedish rural communities is that geographical isolation and migration are not the only factors active in determining it. Geographical isolation seems to be determinant in the case of the parish of Muonionalusta, located in the extreme North of the country, where the incidence of first cousin marriages is the highest recorded. The relatively high incidence (1.7 per cent) observed by LARSON (1956) in Börringe demonstrates that other factors are also of importance, since this community has no geographical isolation, is located in the most densely populated part of the country, and has a rather mobile population. Analysis of sociological and economic factors should prove of interest. When it has been possible to estimate the effective population size, it appears that there are rural communities in Sweden where the actually breeding groups are small.

Estimates of isolate size from consanguineous marriages are not very efficient. MORTON (1955) has recently shown that non-randomness in such marriages affects this estimate. For a general discussion of this subject, see BÖÖK (1956 a). The inbreeding coefficient and the estimate of isolate size by »distance» (WRIGHT, 1946) are of more direct use for genetical purposes.

As far as estimation of the incidence of consanguinity in the general population is concerned, the data available are not quite conclusive. However, a decrease in the general rate of consanguinity seems evident

from the data presented in this paper, *i. e.* the higher incidence in the parents of applicants for legal abortion than in the applicants themselves and the decrease in the Swedish nobility. Data from rural communities are not so definite in this respect.

The incidence of parental consanguinity in a sample of hospital patients in Sweden (0.62 per cent) is in agreement with that determined by BELL (1940) in England. The estimated incidence of first cousin marriages in the Swedish sample is 0.49 (if these first cousin marriages are in the same proportion as in the sample of BELL). Taking this figure as a medium estimate, and those for the nobility (1921—1930) and for applicants for legal abortion in towns as an upper and lower limit, we can tentatively give a range of 0.80—0.40 per cent for the incidence of first cousin marriages in the general population of Sweden.

Acknowledgment. — I am indebted to professor J. A. BÖÖK for his assistance and advice in the preparation of this paper.

SUMMARY

(1) The available information on the incidence of consanguineous marriages (with emphasis on first cousin marriages) in Sweden has been presented.

(2) Estimates have been obtained by following the genealogies of single families in parish books of six rural communities of different geographical and social constitution. The incidence of first cousin marriages ranges from 6.8 to 0.57 per cent. The highest rate is observed in a geographically isolated locality in the North. An incidence of 1.7 per cent in a densely populated locality of South Sweden with no geographical isolation shows that other than purely geographical factors are active in determining the rate of consanguinity.

(3) The consanguinity rate in a peculiar type of »isolate», the Swedish nobility, has steadily decreased in a period of a hundred years (1820—1930).

(4) Consanguinity rates in samples of the general population, such as applicants for abortion and hospital patients, are also presented. The incidence of first cousin marriages in the general population of Sweden is tentatively estimated to be between 0.80 and 0.49 per cent.

Addendum

Professor ESSEN-MÖLLER informs me (exchange of letters in 1956) that he estimated the incidence of first cousin marriages to be 0.6 per

cent in a rural locality of South Sweden (Malmöhus län). This locality numbered 2550 inhabitants in 1947. All marriages contracted by any inhabitant living within the area have been considered. A first report on an extensive medical census in this area has been published. (ESSEN-MÖLLER, E. — Individual traits and morbidity in a Swedish rural population. Acta psychiat. neurol. scand. supp. 100, 1956).

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THE GEOGRAPHICAL DISTRIBUTION OF ACCESSORY CHROMOSOMES IN CENTAUREA SCABIOSA

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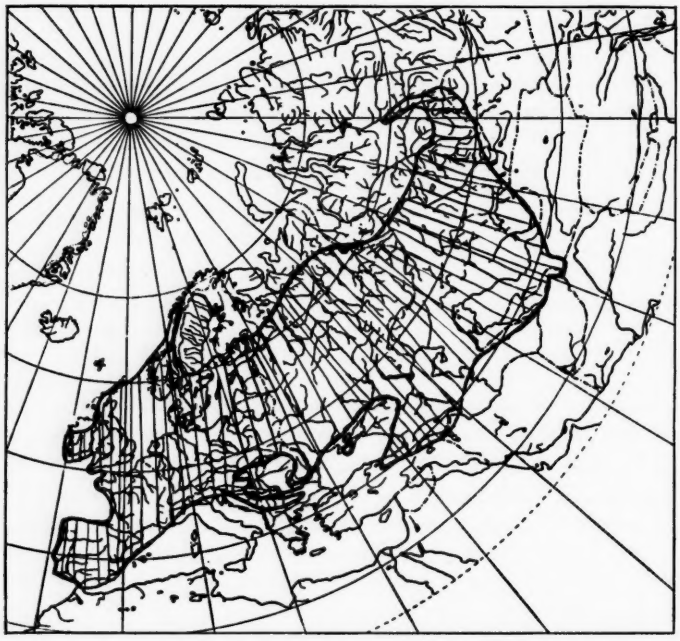
I. INTRODUCTION

THE cytological behaviour and mode of transmission of accessory chromosomes in *Centaurea scabiosa* have been described in two previous publications (FRÖST, 1956, 1957). The present paper concerns the geographical distribution and frequency of plants with accessory chromosomes. Natural populations from Scandinavia and Finland were mainly considered but collections were also investigated from certain parts of England, France, Belgium, Luxembourg, Germany, Austria, and Yugoslavia.

The distribution of *C. scabiosa* in Europe and Asia is shown in Map 1. The species occurs throughout Europe except in its most southern and northern parts and extends far into Siberia; it has also been introduced into North America. Map 7 gives a more detailed picture of the distribution of the species in Scandinavia and Finland.

In most areas *C. scabiosa* may be considered a colonist — but as a very old one. The species is probably native only in the Russian steppe-districts (*cf.* STERNER, 1922, p. 293). *C. scabiosa* is to a very high degree dependent on man for its spread and often grows on dry and sunny localities more or less transformed by human activities such as pastures, field paths, hillsides and road-sides.

Within some areas, especially in the mountainous districts on the Balkan peninsula and also in central Europe, there are some forms of the species which deviate morphologically considerably from the ordinary *vulgaris*-type. These forms have been given special names by different authors as ssp. *Fritschii*, ssp. *spinulosa*, ssp. *badensis*, ssp. *Sadleriana* and ssp. *alpestris*. The species type generally found in different parts of Europe is, however, the ordinary type (*vulgaris*). This type is also the only one occurring in Scandinavia—Finland. However, in

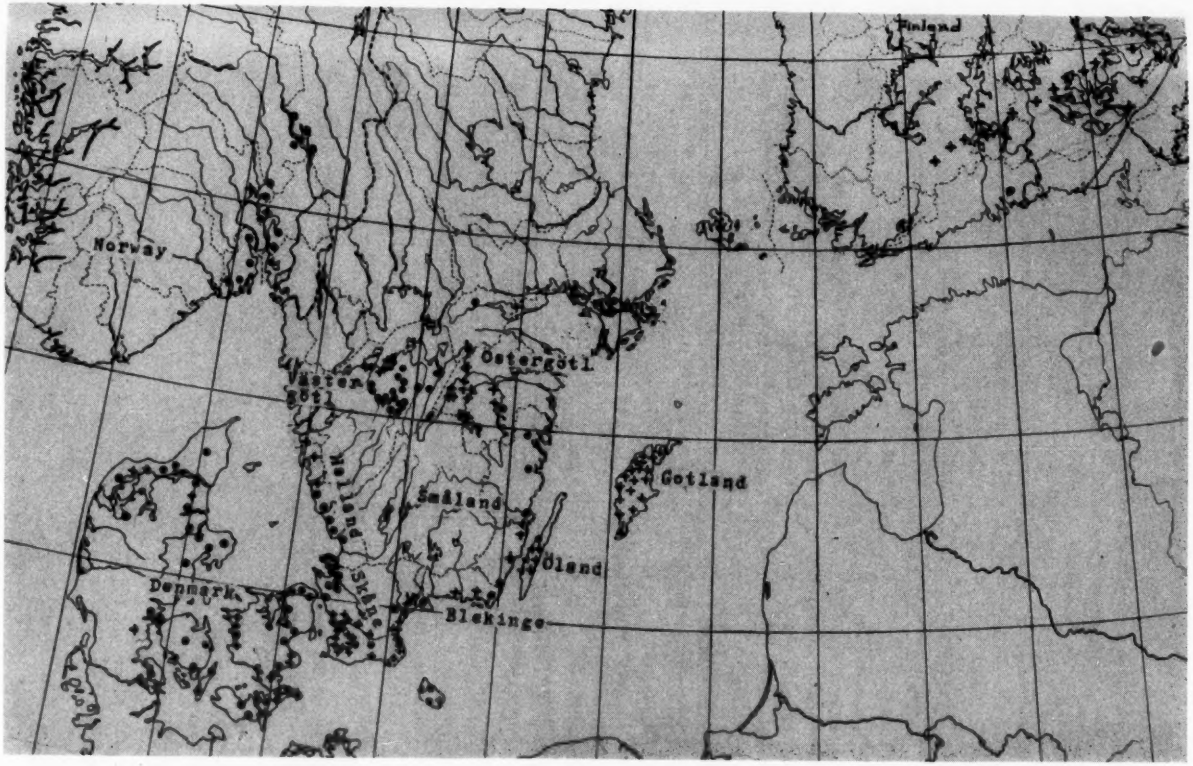


Map 1. The distribution of *Centaurea scabiosa* in Europe and Asia. (The map from E. HULTÉN, unpublished.)

Yugoslavia one of the investigated populations probably belonged to ssp. *Fritschii* and the material collected in the Alps probably belongs to ssp. *alpestris*.

II. MATERIAL AND METHODS

In the aggregate, seeds were collected from 304 natural populations. In each population, one or two ripe heads per plant were taken from a fairly large number of plants, the number, however, being adjusted to the size and range of the population in question. After threshing, the seeds were mixed and a sample of 70 to 100 seeds from this mixture were germinated in petri dishes. From each seedling the first root was fixed in chrome-acetic-formalin (MÜNTZING's modification of NAVASHIN's fluid, MÜNTZING, 1933), embedded in paraffin, sectioned at 16 μ and stained in crystal violet. From about 60 populations, however, the seeds were sown in pots and later transplanted into the field. This material has mainly been used for cytological studies and crossing experiments.



Map. 2. The location of sampled natural populations of *Centaurea scabiosa* in Scandinavia and Finland. (+ = populations with accessory chromosomes; ● = populations without accessory chromosomes.)

TABLE 1. *Frequency of accessory chromosomes in natural populations*

(The different areas are

	Area	Number of plants with respective number of accessory								
		0	1	2	3	4	5	6	7	8
1	Western Scania	1027	103[1]	77	33[2]	15	10	7	11	4
2	Halland	270	16	10	4	5	8	1		1
3	Västergötland	1090	6	7	11	5	5	4	5	2
	Total western Sweden	2387	125	94	48	25	23	12	16	7
4	Närke	46								
5	Uppland	36								
6	Östergötland	296	80[3]	77[4]	56[5]	46[6]	28[7]	18[8]	14[9]	8
7	Eastern Småland	235	19[10]	24[11]	10	5	1			
8	Öland	209	29[10]	27	20[5]	8[12]	7	5[13]	3	2
9	Gotland	279	111[14]	121[15]	95[5+18]	57[16+19]	32[7]	31[17]	18	12
10	Blekinge	83	16[3]	12[20+11]	13[2]	8	6			1
11	Eastern Scania	198	58[21]	55[11]	25[2]	33[22]	18[23]	14	8	7
	Total eastern Sweden	1382	313	316	219	157	92	68	43	30
12	Sjælland	276	16	19	9	2	1	1	2	
13	Laalland, Falster and Mön	138	12	5		1			1	
14	Fyn	150	10	5						
15	Jutland	289	8	5	3	1				
16	Bornholm	57	2	3	2					
	Total Denmark	910	48	37	14	4	1	1	3	
17	Norway	613	6	10	4	5	—	—	1[9]	
18	Finland	396	119[24]	107[20+25]	48[18]	27[6]	12[7]	2	1	2
	Total Scandinavia and Finland	5688	611	564	333	218	128	83	64	39

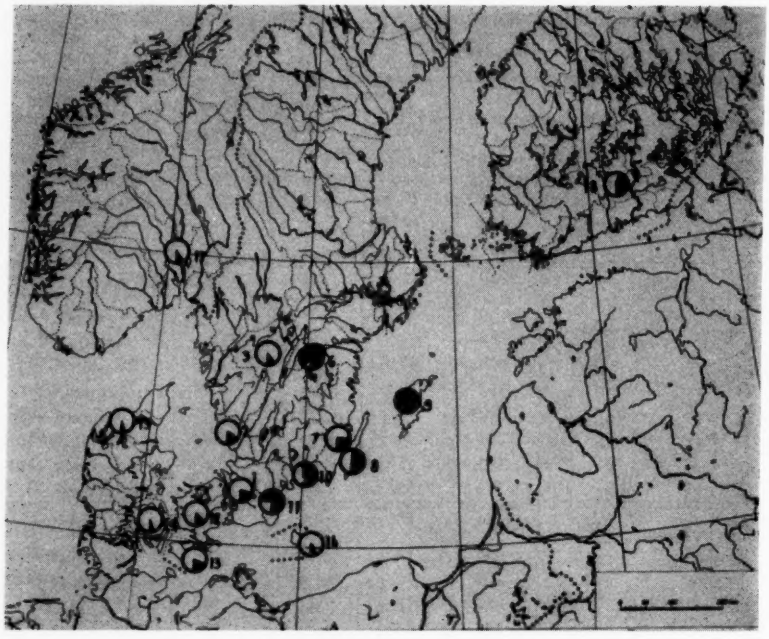
1. Two plants had one acc. iso-chr.
2. One plant » » » » and 2 st. acc. chr.
3. Seven plants » » » »
4. Three » » » » » » 1 » » »
5. » » » » » » » 2 » » »
6. Four » » » » » » 3 » » »
7. One plant » » » » » 4 » » »
8. Two plants » » » » » 5 » » »
9. One plant » » » » » 6 » » »
10. » » » » » »
11. » » » » » » » 1 » » »
12. » » » » » » » 3 » » »
13. » » » two » » » 4 » » »

of Centaurea scabiosa. Collections made in Scandinavia and Finland.

numbered according to Map 3.)

chromosomes 9	10	11	12	13	14	15	16	Total number of plants	% plant with acc. chr.	Number of populations with acc. chr.	without acc. chr.	total	Average number of acc. chr. per plant
	1							1288	20	19	16	35	0.45
								315	14	3	5	8	0.37
1	—	1						1137	4	5	23	28	0.24
1	1	1						2740	13	27	44	71	
								46	—	—	1	1	0
								36	—	—	1	1	0
1	7				1	1		633	53	14	1	15	1.79
								294	20	5	2	7	0.49
1	1							312	33	6	1	7	1.00
4	3		1				1	765	64	18	—	18	2.03
1					1			141	41	3	1	4	1.13
	2							418	53	13	—	13	1.50
7	13		1		2	1	1	2645	48	59	7	66	
								326	15	6	11	17	0.24
								157	12	4	5	9	0.44
								165	9	1	6	7	0.06
								306	6	4	13	17	0.07
								64	11	2	2	4	0.17
								1018	11	17	37	54	
								639	4	3	12	15	0.21
	1							715	45	15	1	16	0.92
8	15	1	1	1	2	1	1	7757	27	121	101	222	

14. Four plants had one acc. iso-chr.
15. Five » » » » » and 1 st. acc. chr.
16. Three » » » » » 3 » » »
17. One plant » » » » 5 » » »
18. » » » two » » 1 » » »
19. » » » » » » 2 » » » +a very small fragment
20. Two plants » » » »
21. Five » » one » »
22. Two » » » » » 3 » » »
23. » » » » » » 4 » » »
24. Fifteen » » » » »
25. Seven » » » » » 1 » » »



Map 3. Average number of accessory chromosomes per plant within different areas in Scandinavia and Finland (*cf.* Table 1).
A solid black circle represents an average of 2 accessory chromosomes per plant.

III. NATURAL POPULATIONS IN SCANDINAVIA AND FINLAND

In Scandinavia and Finland 222 natural populations were studied and the chromosome number determined in 7757 plants (Table 1, and Maps 2 and 3). These collections were made mainly during the years 1950—54. The data for the different Swedish populations have been grouped according to province. The provinces can be considered as fairly good natural units as the collections have most often been made on the plain districts within the respective provinces. However, the easternmost area of the province of Scania has been separated from the rest. The reason for this will be discussed later. The location of the populations within the different provinces appears on Map. 2. The data from populations sampled in Norway in the vicinity of Oslo and in the southern interior of Finland have been combined for the respective countries. The populations in Denmark are more evenly spread over the

TABLE 2. *Statistical analysis of the data from Table 1 based on the mean values for the individual populations.*

	$\bar{X}$ acc. chr. per plant	Sum of squares	Degrees of freedom	Mean square
Between main groups		61.640	1	61.640
Within the western subordinate group	0.279	64.763	139	0.466
Within the eastern subordinate group	1.367	124.299	82	1.516
Total within main groups		189.062	221	0.855
Between subordinate groups		27.243	16	1.703
Within subordinate groups		161.819	205	0.789
Total	0.684	250.702	222	1.129

Quotient [1] $= \frac{61.40}{1.703} = 36.054$ $\quad P < 0.001$

country and have for the sake of comparison been divided into 4 groups (*cf.* Table 1 and Map 3).

As may be seen, plants with accessory chromosomes are present in all the areas studied. The frequency of such plants, however, is markedly different in the different areas. Thus, the populations from Norway, Denmark, and western Sweden (the provinces of Västergötland and Halland) have on an average a very low frequency of plants with accessory chromosomes whereas the frequency is high in Finland, on the islands of Öland and Gotland, and in the provinces of Östergötland and Blekinge in eastern Sweden. A similar east-west distribution is also found within the province of Scania (*cf.* Map 9 and Table 7).

The differences in frequency of accessory chromosomes between the various areas were tested by means of an analysis of variance where the mean values for the individual populations were used as variates. The east Swedish provinces and Finland form one of the two main groups, the other one being constituted by the west-Swedish provinces, Denmark and Norway. The subordinate groups are the different provinces or other smaller geographical areas (see Table 2).

As seen from Table 2, the difference between the eastern and western

[1] As there are significant differences between subordinate groups, the mean square for »between main groups» has to be divided by the mean square for »between subordinate groups».

groups is highly significant. When judging this significance it must be remembered that the different subordinate groups are not equally well sampled. In the eastern group the populations are mostly located in the eastern part of the province of Scania, the province of Östergötland, the island of Gotland, and Finland. On the other hand, the eastern parts of the province of Småland and the province of Blekinge were not investigated to the same extent. Also in the western group, certain areas are better represented than others. Out of the 139 populations investigated, 63 were collected in the province of Västergötland and the western part of the province of Scania.

Besides the marked differences in frequency of accessory chromosomes between different parts of Scandinavia and Finland, the very high frequency of accessories within most of the eastern areas is of special interest. As can be seen from Table 1, the average frequency of plants with accessory chromosomes in the province of Östergötland, the eastern part of the province of Scania and on the island of Gotland are 53, 53, and 63 per cent, respectively. Also, in Finland the frequency of plants with accessory chromosomes is remarkably high. The data from the island of Gotland (Table 3) are given as an example of the frequency of plants with different numbers of accessory chromosomes within individual populations.

To find a possible explanation of the very marked differences in frequency of accessories between areas and populations, attempts were made to correlate these differences with the average ecological conditions in different areas also taking into consideration the average size of the populations within the different areas. The possible immigration-roads to Scandinavia—Finland and the manners in which *C. scabiosa* spreads will also be discussed.

IV. ECOLOGICAL AND HISTORICAL FACTORS

a. Macro-climatic relations

When analysing the different ecological factors which influence the species distribution some agreement was found between certain climatic factors and frequency of accessory chromosomes.

From Map 4, showing the annual precipitation in Scandinavia and Finland, it can be seen that the precipitation is generally higher in the western areas than in the eastern. The precipitation is thus rather high in the southern part of Norway, the west-Swedish province of Halland,

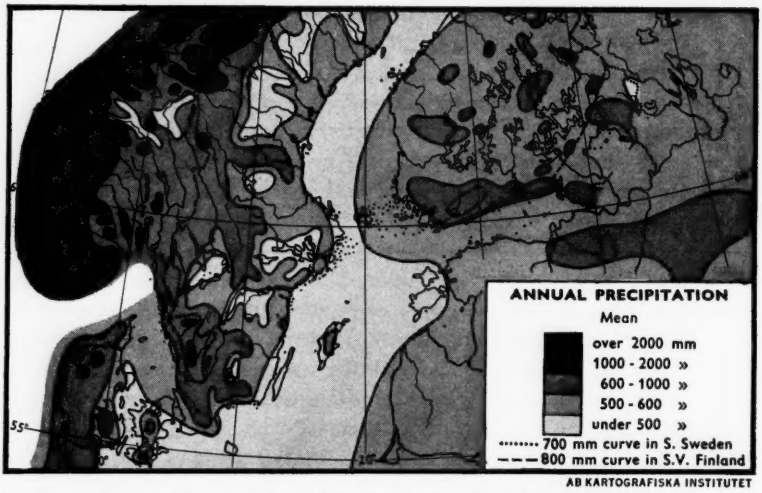
TABLE 3. *Frequency of accessory chromosomes within the individual populations on the island of Gotland.*

TABLE 3. *Frequency of accessory chromosomes within the individual populations on the island of Gotland.*

Sample Nos.	Number of plants with respective numbers of accessory chromosomes																	Total number of plants	Average number of acc. chr. per plant
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
Sg- 97	5	3	6	4	5	6	2	3	—	2								36	3.56
» 98	13	8	11	7	5	3	2	1	3				1					54	2.61
» 99	22	11[1]	13[2]	7[3]			1[4]											54	1.19
» 100	35	7	2	2														46	0.37
» 101	1	6[5]	15[6]	11	11	5	8	1	3	1	1							63	3.75
» 102	4	15	12	7	7	1	1	1	1									49	2.33
» 103	4	3	8	9	3	7	5	2	1								1	43	3.74
» 104	9	4	13	5[7]	3	1	2	2	1	1								41	2.56
» 105	5	5	2	11[7]	3	2	2	1	1									32	2.84
» 106	6	10	7	9	4[8]	3	—	2	1									42	2.48
» 107	24	9	8[6]	4	2[9]	—	1											48	1.08
» 108	2	1	4	3	7[10]	3[11]	1				1							22	3.50
» 109	23	3	3	7[7]	1													31	0.52
» 110	30	3	5	2	1													41	0.56
» 111	20	9	4	4	1													38	0.87
» 112	31	3	1															35	0.14
» 113	40	7	4[6]	4	1	—	3											59	0.83
» 114	5	4	3	5	3	1	3	5	1	—	1							31	3.65
Total	279	111	121	95	57	32	31	18	12	4	3	—	1	—	—	—	1	765	2.03

1. Three plants had 1 acc. iso-chr.
2. Two » » 1 » » and 1 st. acc. chr.
3. One plant » 2 » »
4. » » » 1 » » » 5 » » »
5. » » » 1 » »
6. » » » 1 » » » 1 » » »
7. One plant had 1 acc. iso-chr. and 2 st. acc. chr.
8. » » » 1 » » » 3 » » »
9. » » » 2 » » » 2 » » » [1]
10. Two plants » 1 » » » 3 » » »
11. One plant » 1 » » » 4 » » »

[1] and a very small fragment.

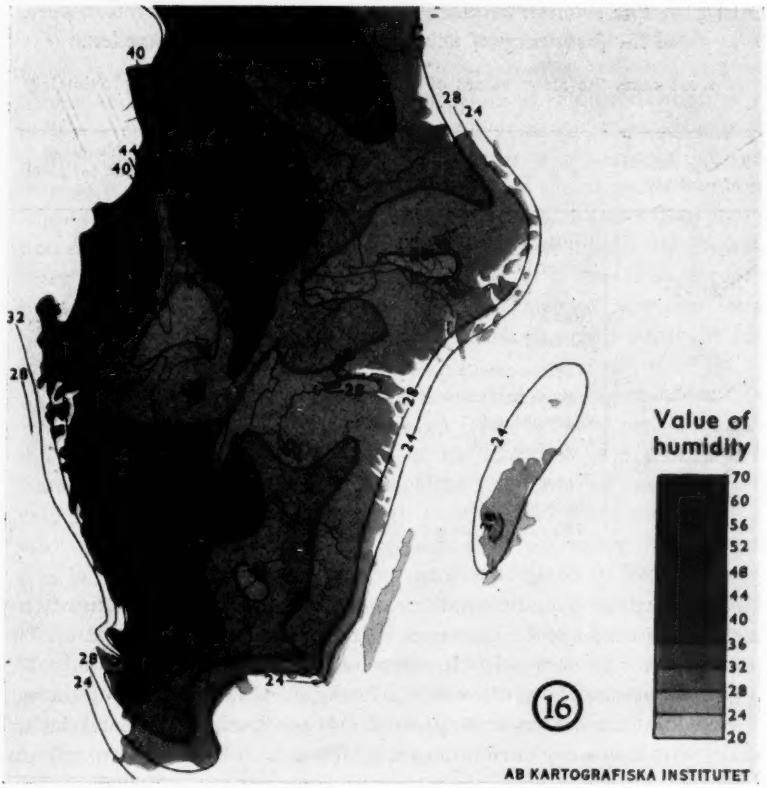


Map 4. The annual precipitation in Scandinavia and Finland.
From E. HULTÉN: Atlas över växternas utbredning i Norden (Atlas of the distribution of vascular plants in N. W. Europe).

and the most north-western part of the province of Scania. With the exception of the eastern and the southern part of the island of Zealand, also in Denmark, the precipitation is relatively abundant. On the other hand, the lowest precipitation ($<$ 500 mm) is particularly found in the eastern part of Sweden, namely, in the province of Östergötland, in the eastern part of the province of Småland, and on the islands of Gotland and Öland. Thus, in areas with abundant rainfall the frequency of accessory chromosomes is generally low whereas the opposite is true for areas with low precipitation. On the other hand, there are areas such as the southern interior of Finland, the west-Swedish province of Västergötland and the larger part of the province of Scania, which have small or no differences in annual precipitation but which have large differences in frequency of accessory chromosomes.

Nevertheless, of greater ecological significance than the precipitation is the humidity of the climate. Map 5 shows the humidity values for the vegetation period in Sweden. The frequency of accessory chromosomes in different areas and the respective humidity values are compared in Table 4.

As may be seen, there is an agreement between the value of humidity and frequency of accessories in Sweden. The frequency of accessory



Map 5. Humidity during the growing season in Sweden. The humidity is calculated according to the formula of MARTONE: $M_v = \frac{365}{n_v} \cdot \frac{N}{10 + T_v}$, where M_v = humidity during the growing season, n = length of growing season and T = mean temperature during the same period. From Atlas över Sverige.

chromosomes is, in general, high in areas with low humidity. The agreement between frequency of accessories and humidity seems thus to be somewhat more linear than the agreement between frequency of accessories and annual precipitation.

Unfortunately, it has not been possible to obtain data of the same type for Norway, Denmark, and Finland. For larger areas of Denmark it may be assumed that the humidity is, on the whole, higher than it is in south-eastern Sweden. There are, however, some areas in Denmark

TABLE 4. *The relation between humidity during the vegetation period and the frequency of accessory chromosomes in Sweden.*

(In some cases the mean values of acc. chr. per plant represent approximately the given areas.)

Average value of humidity	Area	Mean value of acc. chr. per plant (cf. Table 1)
20—28	South-western Scania	(0.63)
	Eastern Scania	1.50
	» Småland	0.49
	Öland	1.00
	Gotland	2.03
28—32	Western Scania (except the most north-western part)	(0.63)
	Östergötland	1.79
32—36	Västergötland	0.24
36—40	The north-western part of Scania	0.06
	Halland	0.37
	(The central parts of Västergötland)	(0.24)

which have drier climatic conditions, *e.g.*, the eastern and south-western parts of the island of Zealand and the most eastern part of Jutland. The populations with accessory chromosomes in Denmark were mainly obtained in eastern and south-western Zealand, while the two populations collected in the easternmost part of the peninsula of Jutland lacked plants with accessory chromosomes (*cf.* Map. 2). The number of populations from the drier areas are too small to permit any conclusions.

The macro-climatic condition in Norway is difficult to estimate because of the topographic conditions. Norway is a very hilly and mountainous country which makes the micro-climatic influences very strong (*cf.* SJÖRS, 1956). The mean annual precipitation is, however, higher than in eastern Sweden.

The situation in Finland is more problematic. The mean annual precipitation in the southern interior is only about 500—600 mm. If we calculate the mean annual humidity, it is, however, evident that the humidity must be rather high because of the low average temperature. As the frequency of accessory chromosomes is high in Finland, it thus seems to constitute an exception to the rule. Nevertheless, other climatic differences as, for example, the more continental character of the climate may influence the situation in Finland.

b. Macro-edaphic relations

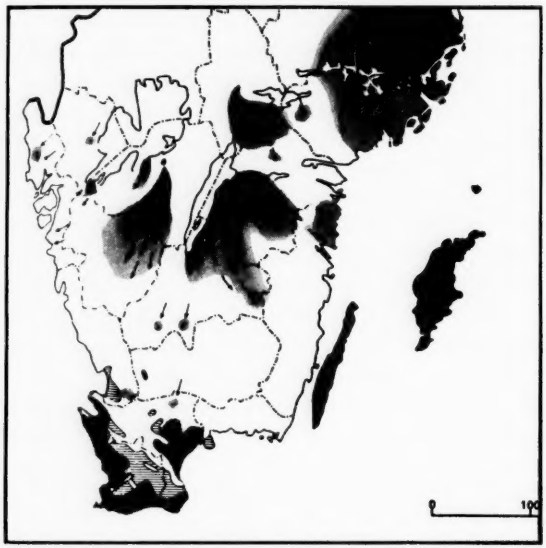
With respect to edaphic conditions, it has not been possible to demonstrate any pronounced differences between areas with low and high frequencies of accessory chromosomes. Maps of the distribution of the different soil types of arable land as well as maps of calcareous soil distributions have been compared with the range of accessory chromosomes. Since the classification of the soil types corresponds to macro-edaphic conditions, a more careful analysis for each particular population might have given a different result. *C. scabiosa* generally grows on drier sites with light and sandy soils although it is also to be found on light morain clays. On heavy clay soils the species is rarely found. Plants growing in areas where heavy clay soils dominate generally grow in »islands» of lighter soils.

Areas where light sandy soils dominate or are very common are found within several of the districts with high frequencies of accessory chromosomes such as the eastern part of the province of Scania and the islands of Öland and Gotland. These light soils naturally emphasize the arid conditions within these districts. On the other hand sandy soils are also common in areas with low frequencies of accessory chromosomes, *e. g.* in the southern part of the province of Halland in Sweden and on the peninsula of Jutland in Denmark.

The distribution of Swedish calcareous soils may be seen in Map 6. Most of the sampled populations were growing in areas with calcareous soils. The same also holds true for Denmark and Norway. The areas investigated in Finland, on the other hand, are characterized by sandy archean soils which are poor in lime.

c. Conclusions

It is, however, difficult to find any general agreements in the environmental conditions for the species in Finland compared with those generally met with in south-eastern Sweden. Nor is it easy to contrast them with the conditions in western Sweden, Denmark and Norway. It may be assumed that the conditions for the species in some parts of eastern Sweden, *e. g.* the islands of Gotland and Öland and some parts of eastern Scania are more optimal than in other areas. Although in the areas just mentioned, *C. scabiosa* is more common than elsewhere in Scandinavia and Finland, it is unlikely that the species is native even there. As has earlier been pointed out, *C. scabiosa* is, according to STERNER (1922), native only in the Russian steppe-districts.



Map 6. Extent of calcareous soils in southern Sweden. From Atlas över Sverige.

The conditions in Finland can scarcely be considered as suitable for the species as those obtaining in eastern Sweden. The southern interior of Finland is an area with sandy archean soils, which are comparatively rich in pine forests. However, the species is fairly common here (growing in pastures or on hillsides and road-banks) and the populations are with a few exceptions large. One difference between Finland and especially the western areas investigated, as previously pointed out, is the continental character of its climate. This climate character is among other things expressed by larger differences in summer and winter temperatures. The mean temperature for January (— 7° to — 9° C) is, in general, lower than any other of the areas investigated while the mean temperature for July (+17° C) is about the same as for southern Sweden. The mean July temperature in Finland is even higher than in the Swedish province of Västergötland, the Danish peninsula of Jutland, and the Danish island of Fyn.

The climate in south-eastern Sweden also possesses some continental features. The low humidity on the island of Gotland and Öland, in the southern and eastern parts of Scania, and in the most eastern part of Småland is according to HESSELMAN (1932) rather unique and is

generally encountered only in the Russian steppe-districts. Also, the distribution of the annual precipitation shows a continental character, often being low during the spring and the beginning of the summer. It may also be pointed out that the continental elements of the Scandinavian flora have their only or main distribution within these east-Swedish areas.

In south-western Sweden, Denmark, and Norway, on the other hand, the precipitation is in general more uniformly distributed over the months of the year and the climate is more oceanic in character.

This more or less strong difference as to continentality and oceanity between Finland and south-eastern Sweden, on the one hand, and the western parts of Sweden, Norway, and Denmark, on the other, might perhaps be important in the distribution of accessory chromosomes.

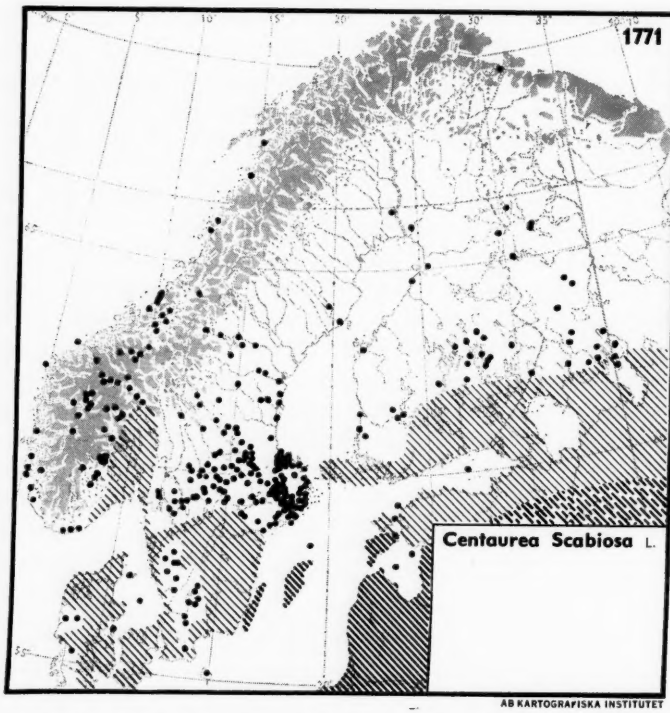
From the above account it is evident that there are average differences in the mean ecological conditions for the species between the investigated areas. These differences indicate that the variation in frequency of accessories between the various areas is a result of different selective values of plants with and without accessory chromosomes under different environmental conditions.

d. Historical factors

Historical factors and chance distribution of the accessories must also be considered. To decide on the importance of these factors, some knowledge of the distribution of the species within the different areas is needed.

In Map 7 the distribution of *C. scabiosa* in Scandinavia and Finland is given. The species appeared, however, to be less common in some areas than is indicated by the map. These areas are the northern and eastern parts of the province of Småland (except the easternmost part), the southern part of the province of Halland, and the southernmost parts of Finland. It appears from the map that the distribution of *C. scabiosa* in southern Sweden is not continuous. The species occurs rarely in the western part of the south-Swedish Highland. The gap in the distribution of the species between eastern and western Sweden may also include Lake Vättern together with the forest district round the lake (*cf.* Map 8).

It is valuable in judging historical factors as a cause for the differences in frequency of accessory chromosomes to know whether these areas act as a barrier preventing the spread of the species. As it is probable

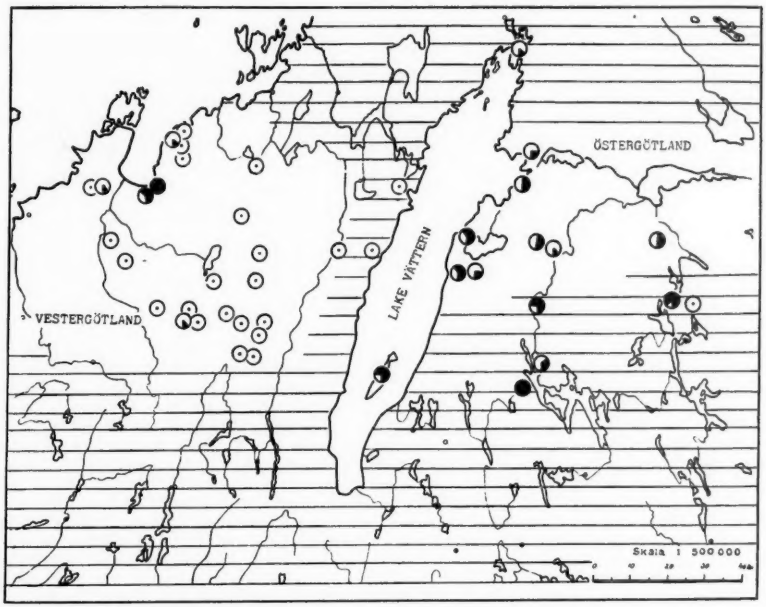


Map 7. The distribution of *Centaurea scabiosa* in Scandinavia and Finland. Heavy lines indicate that the species is common or very common, fine lines that it is fairly common and dots shows isolated instances.
From E. HULTÉN, Atlas över växternas utbredning i Norden (Atlas of the distribution of vascular plants in N. W. Europe).

(*cf.* HULTÉN, 1950) that *C. scabiosa* invaded Scandinavia and Finland from Russia, the Baltic countries, and Denmark it is possible that the different areas received material differing with respect to the average genotypic constitution as well as to the frequency of accessories. In order to study how such assumed historical factors may have influenced the present-day situation, more extensive collections were made in the provinces of Östergötland, Västergötland, and Scania.

As may be seen from Tables 5 and 6 and Map 8, marked differences in the frequency of accessory chromosomes exist between sampled areas within *the provinces of Västergötland and Östergötland.*

If it is assumed that the populations of *C. scabiosa* in the province of



Map 8. The distribution of plants with accessory chromosomes in the provinces of Västergötland and Östergötland. The forest districts are designated by horizontal lines and unlined areas mark roughly the extent of the Västgöta and Östgöta plain districts. The populations are represented by circles which are divided according to average number of accessory chromosomes per plant.

- ⊙ without acc. chr.
- ◔ <0.5 » » per plant
- ◔ 0.5—1.0 » » » »
- ◔ 1.0—1.5 » » » »
- ◑ 1.5—2.0 » » » »
- ◑ 2.0—2.5 acc. chr. per plant
- ◕ 2.5—3.0 » » » »
- ◕ 3.0—3.5 » » » »
- ● >3.5 » » » »

Västergötland originally lacked accessory chromosomes as a result of historical factors or by more chance, the possibility for these differences to persist depends upon the degree of migration of plants with accessory chromosomes. The geographical barrier between the Östgöta and the Västgöta plains can scarcely be considered a hindrance completely preventing the natural migration, since the species also occurs, although rarely, in the districts between the plain districts. *C. scabiosa* is, however, to a large degree, spread by man and the species has great possibilities to be spread over long distances. The occurrence of the two populations with very high frequencies of accessories in the vicinity of Kinnekulle mountain might be explained in this manner. Whether

TABLE 5. *Frequency of accessory chromosomes within the individual populations in the province of Västergötland and Östergötland.*

Area	Sample Nos.	Number of plants with respective numbers of accessory chromosomes															Total number of plants	Average number of acc. chr. per plant
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Västergötland	S- 71	29	1	—	1													31	0.13
	71b	27																27	0
	72	39																39	0
	73	37																37	0
	75	47																47	0
	76	38	2	1	1	1												43	0.26
	77	40																40	0
	78	36																36	0
	79	28																28	0
	80	34																34	0
	81	36																36	0
	158	10	2	3	3	2	3	2	1									26	2.27
	159	7	—	2	6	2	2	2	4	2	1	—	1					29	4.00
	160	39	1	1														41	0.57
	161	49																49	0
	162	29																29	0
	163	54																54	0
	164	57																57	0
	165	40																40	0
	167	32																32	0
	168	51																51	0
	169	45																45	0
	170	43																43	0
	172	46																46	0
	174	35																35	0
	175	52																52	0
	176	62																62	0
	177	48																48	0
Total		1090	6	7	11	5	5	4	5	2	1	—	1					1137	
Östergötland	S- 82	52	3	2	2													59	0.22
	83	27	7	3	6	3												46	0.94

	176	62																	62	0
	177	48																	48	0
Total		1090	6	7	11	5	5	4	5	2	1	—	1						1137	
Östergöt-	S- 82	52	3	2	2														59	0.22
land	83	27	7	3	6	3													46	0.94
	84	13	8	4	2	5	2	1	1										36	1.81
	85	16	9	4	8	4	6	—	2										49	2.06
	86a	10	3	6	2	3	1					2							27	2.15
	86b	29	4	3	3	1													40	0.58
	87	17	7[1]	10	7	1[2]	2[3]	1	2										47	1.75
	88	30	—	1	1	—	1												33	0.30
	89	—	—	2	2	5	8	10	3	3	1	2							36	5.67
	90	21	12[4]	6	6	6[5]	2	3[6]	4[7]	2	—	3	—	—	—	1	1		67	2.94
	91	19	15[8]	12[9]	6[10]	2[11]	1	2	1										58	1.53
	92	3	3	15[12]	7	7	4	1	1	3									43	3.19
	92b	42																	42	0
	98a	16	6	5	2	3													32	1.06
	60	1	3[1]	5	2	6	1												18	2.67
Total		296	80	77	56	46	28	18	14	8	1	7	—	—	—	1	1		633	

1. One plant had 1 acc. iso-chr.
2. » » » 1 » » and 3 st. acc. chr.
3. » » » 1 » » » 4 » » »
4. Three plants » 1 » »
5. Two » » 1 » » » 3 » » »
6. » » » 1 » » » 5 » » »
7. One plant had 1 acc. iso-chr. and 6 st. acc. chr.
8. Two plants » 1 » »
9. » » » 1 » » » 1 » » »
10. Three » » 1 » » » 2 » » »
11. One plant » 1 » » » 3 » » »
12. » » » 1 » » » 1 » » »

TABLE 6. *Statistical analysis of the data from Table 5.*

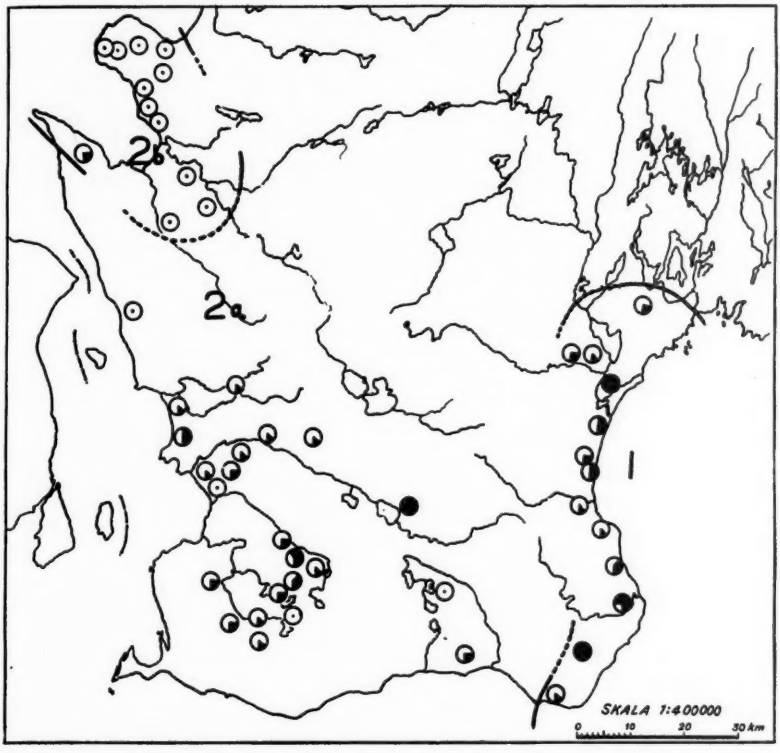
	$\bar{X}$ acc. chr. per plant	Sum of squares	Degrees of freedom	Mean square
Between V. and Ö.		23.459	1	23.459
Within V.	0.240	19.620	27	0.727
» Ö.	1.790	29.957	14	2.140
Total within:		49.577	41	1.209
Total:		73.036	42	

$$\text{Quotient} = \frac{23.459}{1.209} = 19.404 \qquad P < 0.001.$$

these population were old or newly established is difficult to decide but one of them was growing on a dry pasture, which had earlier been cultivated. Thus, it is possible that this population was relatively young. The other population was growing close to a gravel-pit. The distance between these two populations was only about 3 english miles, and they might therefore have a common origin. Thus, if we assume that in the province of Västergötland *C. scabiosa* originally lacked accessory chromosomes, these two populations with high frequencies of accessories as well as three other populations with accessory chromosomes demonstrate that there is a certain migration of plants with accessory chromosomes into the province of Västergötland. In the province of Östergötland as well as in the province of Västergötland *C. scabiosa* is known to be a very old colonist. If the differences in accessory chromosomes date from the time of migration of the species to the respective provinces and these differences do not depend on selective causes, it is surprising that these initial differences still persist, even if the migration of plants with accessory chromosome is relatively slight.

If, on the other hand, the differences are of a relatively recent date they are easier to explain. Yet from the investigation of the geographical distribution of the accessory chromosomes in different parts of Europe, it has been shown that accessory chromosomes of the same morphological type are spread over the whole distribution area of the species. It can thus rather safely be concluded that accessory chromosomes are of very old origin and also be supposed that in the populations in eastern Sweden they are of the same age as the species itself.

Instead of attributing the differences in frequency of accessories be-



Map 9. The distribution of plants with accessory chromosomes in the province of Scania. The province is divided into three parts as in Table 7. The populations are represented by circles with the same divisions as Map 8.

tween the Östgöta and the Västgöta plains to historical factors or to mere chance distribution, it is considered that plants with accessory chromosomes prevail in certain areas as a result of an interaction between the environmental conditions and the genotypical constitution of the plants. An indication that this might be the case is the differences in humidity. As was pointed out earlier (*cf.* Map 5), the climate in the province of Västergötland is generally more humid than that in the province of Östergötland. These differences are, however, relatively small and to what extent they influence the ecological conditions of the species it is impossible to decide.

TABLE 7. *Frequency of accessory chromosomes within the individual populations in the province of Scania.*

Area	Sample Nos.	Number of plants with respective numbers of accessory chromosomes															Total number of plants	Average number of acc. chr. per plant
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
North-western Scania	S- 40	15															15	0
	41	9															9	0
	42	9	4	4													17	0.71
	44	11															11	0
	45	14															14	0
	145	57															57	0
	146	26															26	0
	179	41															41	0
	180	47															47	0
	181	42															42	0
	182	58															58	0
	183	42															42	0
Total		371	4	4													379	
South-western Scania	S- 38II	12															12	0
	49	32	4	5	1												42	0.41
	50	8	2	4	3	3	4	2	7	3	—	1					37	3.95
	51	25															25	0
	52	10	6	1	1												18	0.61
	130a	43	2	3													48	0.17
	131	38	2[1]														40	0.05
	132	34	4	4	1	2											45	0.51
	133	29	5	4	1												39	0.41
	134	54															54	0
	135	16	4	4					1								25	0.76
	136	14	2	5	6[2]	1	4	3	1	1							37	2.35
	137	30	3	2	1												36	0.27
	138	27	5	6	6	3	1										48	1.08
	139	31	16	4	1												[illegible]	[illegible]
	142	33	3	3	1			1									41	0.44
	143	26	8	7	1	1											43	0.67
																	51	0.73

	136	14	2	5	6[2]	1	4	3	1	1			37	2.35
	137	30	3	2	1								36	0.27
	138	27	5	6	6	3	1						48	1.08
	139	31	16	4	1								41	0.44
	142	33	3	3	1			1					43	0.67
	143	26	8	7	1	1							51	0.73
	144	32	9	6	1	2	1						53	1.55
	150	20	11	9	7	3	.	1	2				59	0.15
	151	52	5	2									43	0.23
	152	35	6	2										
Total		656	99	73	33	15	10	7	11	4	—	1	909	
Eastern Scania	S- 61	—	6	2	5	5	5	5	—	1	—	1	30	3.93
	53	3	5	7	6	4	1	—	2	1			29	2.76
	54	14	4	4	1	1						1	25	1.16
	55	8	1	2									11	0.46
	56	5	1	1									7	0.43
	57	5	—	2	1								8	0.88
	128	21	8[1]	9									38	0.68
	129	15	2[3]	1	2[2]	5[4]	4[5]						29	1.72
	153	35	7[3]	3[6]	2	1	1						49	0.47
	154	33	7	5	—	1							46	0.46
	156	—	4	12	5	7	4	7	5	3			47	4.09
	157	18	10	6	1	7	2	2	1	2			49	2.00
	178	41	3	1	2	2	1						50	0.48
Total		198	58	55	25	33	18	14	8	7	—	2	418	

1. One plant had one accessory iso-chr.
2. » » » » » » and 2 st. acc. chr.
3. Two plants » » » »
4. » » » » » » » 3 » » »
5. » » » » » » » 4 » » »
6. One plant » » » » » 1 » » »

TABLE 8. *Statistical analysis of the data from Table 7.*

	$\bar{X}$ acc. chr. per plant	Sum of squares	Degrees of freedom	Mean square
Between w. Sc. and e. Sc.		10.398	1	10.398
Within w. Sc.	0.454	21.228	34	0.624
» e. »	1.501	21.085	12	1.757
Total within:		42.313	46	0.920
Total:		52.711	47	

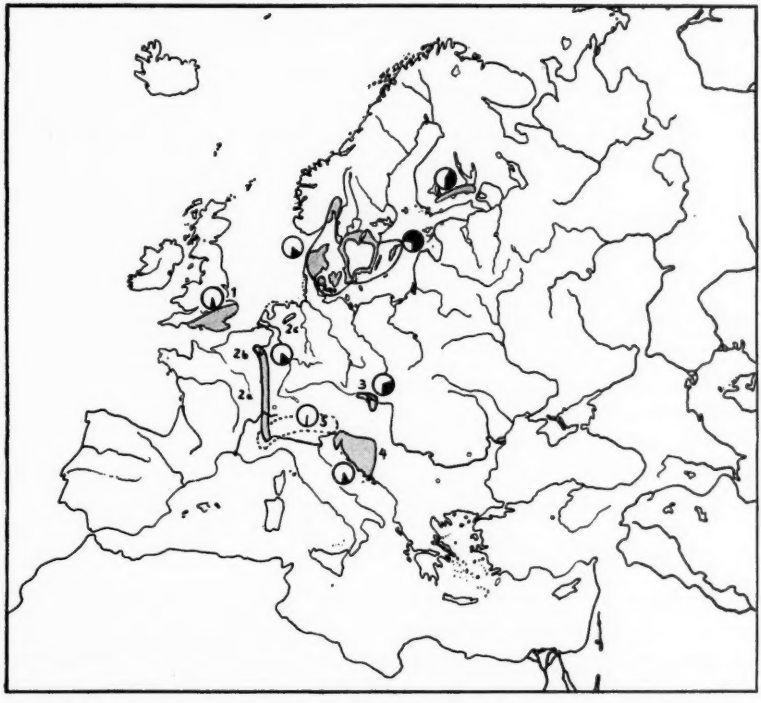
$$\text{Quotient} = \frac{10.398}{0.920} = 11.302 \qquad 0.01 > P > 0.001.$$

The edaphic conditions are rather variable. Most of the populations in the province of Östergötland were collected on an area called Vadstena-plain. However, some of the populations were taken in the northernmost part of the south-Swedish Highland. The populations collected in the province of Västergötland originate mostly from an area called Falbygden and from the vicinity of Kinnekulle mountain. These areas as well as the areas sampled in the province of Östergötland are characterized by calcareous soils. In both the Östgöta and the Västgöta plains, *C. scabiosa* is fairly common and there are no noticeable differences between the two areas in this respect. The populations grew most often on sandy soils or light moraine clays.

A similar difference in frequency of accessories to that between the provinces of Västergötland and Östergötland also occurs between different areas within *the province of Scania.* (Map 9 and Table 7.) The statistical analysis shows that there is a significant difference in frequency of accessories between the eastern and the western parts of the province of Scania (Table 8). This difference, however, is to a very high degree influenced by the populations in the north-western area, which largely lack accessory chromosomes.

As pointed out, the north-western part of the province is more humid than the south-western and eastern parts (*cf.* Map 5).

The macro-edaphic conditions in these different plain districts are variable. In the eastern part of Scania, sandy soils predominate and there are also places with steppe-like soils (cf. SJÖRS, 1956). The sandy soil together with the fairly low precipitation, results in a nearly arid climate in certain regions (*cf.* HESSELMAN, 1932). In the south-western plain district, the edaphic conditions are even more variable. The dominating soil



Map 10. The frequency of accessory chromosomes within different parts of Europe. A solid black circle represents 2 acc. chr. per plant.

1.	England	0.08 acc. chr. per plant	
2 a.	France	0.28 » » » »	0.32 acc. chr. per plant
b.	Belgium—Luxemburg	0.42 » » » »	
c.	Western Germany	0.25 » » » »	
3.	Austria	0.70 » » » »	
4.	Yugoslavia	0.21 » » » »	
5	The Alps	0.01 » » » »	

(Denmark, Norway, western Sweden 0.28, eastern Sweden 1.48 and Finland 0.92 acc. chr. per plant.)

type is here moraine-clay but sandy soils are also common. In the north-western part of the province, also, there are various kinds of soils such as sandy soils and moraine clays. On the Ängelholm-plain, however, heavy sedimentary clays dominate. From this area three populations have been sampled. In the eastern as well as in the south-western parts of the province, the populations were collected in areas which are

TABLE 9. *Frequency of accessory chromosomes*

	Area	Number of plants with respective numbers of accessory									
		0	1	2	3	4	5	6	7	8	9
1	England	615	16	7	1	1					
2a	France	579	47	24	12	9	—	2	1		
2b	Belgium-Luxemburg [1]	226	28[1]	19	4	4	2	2	—	—	1
2c	Western Germany	115	5	3	5	2					
3	Austria	459	37[2]	21[3]	24[4]	21[5]	14	7	4	2[6]	1
4	Jugoslavia	842	28[7]	25[8]	20[9] + [11]	12[10]	3	2			1
5	The Alps	405	1	—	1						
	Total	3241	162	99	67	49	19	13	5	2	3

1. Two plants had 1 accessory iso-chr.
2. One plant » 1 » »
3. » » » 2 » »
4. » » » 2 » » and 1 st. acc. chr.
5. » » » 2 » » » 2 » » »
6. » » » 2 » » » 6 » » »

[1] (1 pop. collected in Luxemburg.)

characterized by very calcareous soils. In the most north-western part of the province, however, where 8 populations were collected, the soils are generally less calcareous.

Within the province of Scania, which is a relatively small area, there are probably no geographical barriers effective enough to isolate the populations between the different plain districts. However, as is seen from Map 7, *C. scabiosa* appears to occur rarely in the northern and central parts of Scania. These areas are forest districts characterized by archean soils. The distribution of the species is, however, continuous through the south-eastern parts.

The results obtained in the province of Scania indicate that plants with and without accessory chromosomes have different selective values under different environmental conditions.

V. RESULTS FROM OTHER PARTS OF EUROPE

The collections outside Scandinavia and Finland were, with the exception of the material from England, made in 1953 (Table 9 and Map 10).

The material from England was collected by Dr. N. O. BOSEMARK in

within different parts of Europe.

chromosomes							Total number of plants	% plants with acc. chr.	Number of populations			Average number of acc. chr. per plant
10	11	12	13	14	15	16			with acc. chr.	without acc. chr	Total	
							640	4	3	9	12	0.08
							674	14	7	7	14	0.28
							286	21	6	—	6	0.42
							130	12	1	2	3	0.25
			1			1	592	22	9	3	12	0.70
							933	10	13	13	26	0.21
							407	0.5	2	7	9[12]	0.01
—	—		1	—	—	1	3662	12	41	41	82	

7. Five plants had 1 accessory iso-chr.
8. Three » » 2 » »
9. Two » » 3 » »
10. » » » 4 » »
11. One plant » 2 » » and 1 st. acc. chr.
12. » of the populations was found to be tetraploid with $2n=40$

1951 and consists of seed material from 12 natural populations of *C. scabiosa*, situated in southern and central England. The populations grew mostly on road-sides and more seldom on natural dry pastures. The frequencies of the accessory chromosomes were low, only 25 plants out of 640 (4 per cent) were found in 3 of the 12 populations.

In France, seeds were taken from 14 localities. Three were situated in the most south-eastern part of the country, while the other 11 originated from eastern and north-eastern France. About 14 per cent of the plants were found to have accessory chromosomes. Accessory chromosomes were also generally found in the material collected in south-eastern Belgium and Luxemburg. About 20 per cent of the plants had accessories and occurred in all six populations investigated. Combining the data from Belgium and Luxemburg with the data from north-eastern France, it seems as if plants with accessory chromosomes are rather common on the plateau of Langrès and in the Ardennes. Out of the total 16 populations, 12 had varying frequencies of plants with accessory chromosomes. However, the frequency of accessories within the individual populations was not particularly high, at least, not if the values are compared with those obtained for the eastern parts of Sweden. The populations in eastern and north-eastern France and

Belgium—Luxemburg grew mostly on sandy hill-sides and pastures which although sandy were often covered by rich vegetation.

In Germany, only 3 populations were collected but as one of them had accessory chromosomes the data also indicate a wide geographical spreading of plants with accessories in Europe.

The highest frequency of accessories outside Scandinavia and Finland was found in the material collected in Austria. The species is common in Austria and the populations which often grow on dry pastures or road-sides were with few exceptions large. The 12 samples were collected in Lower Austria, mainly on the plain districts around Vienna. Out of 592 plants, 133 had a varying number of accessories and were found in 9 of the 12 populations. However, the high average number of accessories (0.70 acc. per plant), was considerably influenced by the high frequencies of accessories in populations Nos. 11 and 13 from south-eastern Austria, which had the mean values of 2.96 and 2.04 acc. chr. per plant, respectively.

In Yugoslavia seeds were collected from 26 populations, the chromosome numbers being determined from 933 plants. It is possible that some of these plants may belong to ssp. *Fritschii* which may well be the case with population Y—1 taken just west of Zagreb. Only seven plants were investigated but of these, 2 plants had 1 and 2 standard acc. chr. Of the 26 samples, 2 were taken in the province of Slavonia, north of the river Sava, while the rest were collected in the mountain districts in the provinces of Slovenia, Croatia, Serbia and Dalmatia. The distribution of the accessories in Yugoslavia is also wide. Half of the populations were found to have accessory chromosomes. However, as in western Europe, the frequencies of accessories within the different populations are generally low; and accessories were present only in 10 per cent of the plants studied. No average differences in plants with accessory chromosomes were obtained between the different parts of Yugoslavia: they were rather evenly distributed over the whole area investigated. The ecological conditions for the species in Yugoslavia differed markedly from those investigated in the other parts of Europe. The area also belongs to the southernmost part of the distribution range of the species (*cf.* Map 1). Furthermore, the country differs in being very mountainous and hilly. The sampled populations grew mostly on hillsides or small dry pastures but sometimes also on field paths and road-sides. *C. scabiosa* does not appear to have very suitable ecological conditions there and is less common than in Austria, north-eastern France and south-eastern Belgium.

The populations taken in different parts of the Alps have been tabulated together since the area represents a natural unit. The populations were in general large; they were found in pastures. The plant habit was clearly different from those in other areas as they were small and robust and probably belonged to the ssp. *alpestris*. Out of 9 collections, 4 originate from the Swiss Alps, 3 from the French Alps, and 1 from the Italian and the Austrian Alps, respectively. Most of the populations apparently lack accessory chromosomes. Out of 407 plants, only 2 had accessory chromosomes. In one of the populations most of the plants were tetraploid with $2n=40$. A tetraploid from the Alps has also been reported by BÖCHER and LARSEN (1955).

VI. THE OCCURRENCE OF THE ACCESSORY ISO-CHROMOSOME

Plants with accessory iso-chromosomes (Tables 1 and 9) have been obtained in several natural populations. This chromosome is about double the size of the standard type and is probably derived from the standard type through mis-division (*cf.* FRÖST, 1956, 1957).

However, compared to the standard accessory chromosome the frequency of accessory iso-chromosomes in natural populations is low. From Scandinavia and Finland 104 plants with accessory iso-chromosomes were found which represented only about 1 per cent of all the plants investigated and about 5 per cent of the plants with accessory chromosomes. About the same frequency was obtained from the material collected in areas outside Scandinavia and Finland.

In general, the frequency of plants with iso-chromosomes within the populations is very low and most often the plants have in addition to the iso-type a number of standard accessory chromosomes. However, a few populations have been found where the iso-chromosome is the dominating type, such as pop. no. F—17 from Finland and pop no. J—24 from Yugoslavia. Also, in some populations from the eastern parts of Sweden the majority of plants had accessory iso-chromosomes *e. g.* pop. no. S—125 from the province of Blekinge. The accessory iso-chromosome thus has a wide geographical distribution similar to the standard accessory chromosome.

As the iso-chromosome is obtained in the progeny of crosses between plants with only standard accessory chromosomes (*cf.* FRÖST, 1957) it should be expected that the accessory iso-chromosome would be found

most often within areas where the frequency of the standard type is high. As seen from Tables 1 and 9 this is generally the case.

VII. DISCUSSION

The data from Finland and Scandinavia will first be considered. Plants with accessory chromosomes were present in all the areas investigated, but the differences in frequency between the eastern and the western parts are especially marked. The high frequency of accessory chromosomes found within several areas in the south-eastern parts of Sweden and the southern interior of Finland is especially interesting. Table 1 shows that 74 out of 82 populations from south-eastern Sweden and the southern interior of Finland have varying frequencies of plants with accessory chromosomes but averaging about 50 per cent, which strongly suggests that the accessory chromosomes must be useful in some way. Such an assumption is supported by the mode of inheritance of the accessory chromosomes (FRÖST, 1956). It has not been possible to demonstrate any mechanism for numerical increase of the accessory chromosomes in *C. scabiosa* which could compensate for the meiotic elimination. Thus, if the accessory chromosomes are able to maintain themselves in the populations and reach such high frequencies as, for instance, in the south-eastern parts of Sweden, it must be assumed that there is natural selection for plants with accessory chromosomes. However, other factors may also influence the situation in these areas. Especially in the islands of Gotland and Öland and also in the eastern part of the province of Scania, the spreading and maintainance of the accessory chromosomes are facilitated by the species being very common, the populations being in general large and probably more permanent.

To explain the low frequency of accessory chromosomes in the populations in the western parts of Sweden, Denmark and Norway, different possibilities were discussed. An indication that selective forces have played a large role was the differences in the average ecological conditions between the different areas. The relations between the macroclimatic conditions and frequency of accessory chromosomes can roughly be summarized as follows:

(1) The areas with low numbers of accessory chromosomes, such as, the north-westernmost part of Scania and the provinces of Halland and Västergötland in Sweden, the Oslo-district in Norway and the

greater part of Denmark are in general characterized by high humidity and by more oceanic features.

(2 a) The areas in Sweden with a high frequency of accessories, such as, eastern Scania, the Kalmar-plain in eastern Småland, the provinces of Blekinge and Östergötland and the islands of Öland and Gotland have a more continental climate with lower humidity.

(2 b) The southern interior of Finland with a high frequency of accessories has a continental climate which, however, must be considered as rather humid.

The good correlation between humidity and frequency of accessory chromosomes in Sweden was discussed. Also the conditions in Norway and Denmark were presented and do not seem to deviate from such an argument. As was pointed out, however, an exception to the rule seems to be the situation in Finland. However, the difference as to continentality and oceanity between Finland and south-eastern Sweden, on the one hand, and the western parts of Sweden, Norway and Denmark, on the other, might be important in the distribution of the accessory chromosome.

The significance of the selective forces were discussed, and the possibility that the differences were mainly due to historical factors or to mere chance was also considered. It is, however, impossible to draw any definite conclusions as to the reasons for the differences in frequency of accessory chromosomes between the western and eastern parts of the investigated areas. However, it seems probable that plants with and without accessory chromosomes have, on an average, different selective values in the different areas. Especially the situation in Scania supports the idea that selective forces have been of importance.

The investigated areas in Austria, Belgium, France, England, Yugoslavia and the Alps represent considerable differences as far as ecological conditions are concerned. It is impossible to say whether the differences in frequency of accessories reflect the differences in the environmental conditions or average genotypical constitution of the plants, or whether they are caused by mere chance.

Some observations may, however, be mentioned: for instance, the almost complete absence of accessory chromosomes in the material collected in the Alps and the relatively high frequency found within Austria. In Austria as in Finland, the climate has a continental character. Furthermore, the plants collected in the Alps deviate considerably from the ordinary type of *C. scabiosa* and very probably belong to ssp. *alpestris*.

Also, in the western parts of Europe, many of the populations have a certain frequency of plants with accessory chromosomes, especially in north-eastern France and in Belgium—Luxemburg. The percentage of plants with accessories as well as the average number of accessories per plant in the individual populations are, however, relatively low especially if compared with those obtained in south-eastern Sweden and in the southern interior of Finland. The average frequencies of accessory chromosomes for the different parts of western Europe and Yugoslavia seem to agree reasonably well with the frequencies obtained for different parts of south-western Sweden, Denmark and Norway.

The data obtained from the various areas outside Scandinavia and Finland are based on a relatively small number of samples. It can, however, safely be concluded that the accessory chromosomes in *C. scabiosa* have a wide geographical distribution. It is thus likely that accessory chromosomes are present in populations of *C. scabiosa* over most of its distribution range.

Studies on the frequency and geographical distribution of accessory chromosomes in natural populations have previously been made in *Festuca pratensis* and *Phleum phleoides* (BOSEMARK, 1956 a and b). The results in *Festuca pratensis* are based upon material from natural populations collected in Sweden and England. It was found that the accessory chromosomes occur within all the investigated areas, but as in *C. scabiosa*, there were considerable differences in the frequencies between the different areas. In Sweden, except in the province of Scania, there was a marked correlation between the frequency of accessories and the clay content of the soil so that the highest frequencies were found on soils with a high content of clay and the lowest frequencies on sandy soils. Areas with heavy clay soils are considered to be more optimal for the species. The lack of correlation between soil type and frequency of accessory chromosomes in the province of Scania is considered to result mainly from the influence of cultivation.

BOSEMARK (1956 b), also made an investigation of the frequency of accessory chromosomes in Swedish natural populations of *Phleum phleoides*. There also differences in the frequency of accessories between the different areas were obtained but as the species has a discontinuous distribution in Sweden it was difficult to draw any definite conclusions as to the causes of these differences. However, there are indications of an influence of ecological factors on the distribution of accessory chromosomes within the island of Öland. Especially interesting in *Phleum phleoides* are the high frequencies of accessory

chromosomes in several areas. These values approach the values found in *C. scabiosa.*

Other investigations on accessory chromosomes in natural populations have been carried out in *Clarkia elegans* (LEWIS, 1951), the grasshopper *Trimerotropis sparsa* (WHITE, 1951) and *Trillium erectum* (SPARROW *et al.*, 1952). In all the different materials different populations have different frequencies of accessories. BOSEMARK (1957) has also demonstrated the occurrence of accessory chromosomes in natural populations of a number of grass-species other than *Festuca pratensis* and *Phleum phleoides.* In spite of limited sampling, accessory chromosomes were found in six out of eleven species investigated.

In cultivated material with accessory chromosomes the investigations in maize and rye are the most extensive. RANDOLPH (1941) found that different strains of maize have different frequencies of accessories. Whereas accessory chromosomes are seldom found in commercial strains of dent, pop and flour corn, they are quite common in some strains of sweet corn. LONGLEY (1938) investigated 33 Indian varieties of maize and, although the accessory chromosomes occurred in most of the strains, they were found more frequently in plants with a low number of knobs on the normal chromosomes. In rye there is a pronounced geographical distribution of the accessory chromosomes (MÜNTZING, 1954 and 1957). In cultivated varieties in Europe the frequency of accessories is very low, whereas higher frequencies are found in primitive strains from Turkey, Afghanistan and Iran. In a primitive strain from Transbaikal the percentage of plants with accessory chromosomes was as high as 28. Still more frequent are the accessory chromosomes in the Far East (MÜNTZING, 1957). Seven strains from Korea were investigated and all had high frequencies of accessory chromosomes, some of the strains up to 80—90 per cent. The very high frequencies of accessory chromosomes in rye from Korea are comparable with the frequencies found in *C. scabiosa* in some parts of south-eastern Sweden. MÜNTZING considers that the presence of accessory chromosomes in rye is a primitive trait, and is of importance for further studies of the centre of origin of cultivated rye.

Several suggestions have been made as to the role played by the accessory chromosomes in the population and in the individual plants. An interesting hypothesis has been presented by ÖSTERGREN (1947). He considers that the accessory chromosomes have a detrimental effect on the vigour as well as on the fertility of the individual and only fight for their own existence. The hypothesis is based on the occurrence of a

well developed mechanism for numerical increase of the accessory chromosomes such as that found in several grasses, among others *Secale cereale* (*cf.* MÜNTZING, 1954), *Anthoxanthum aristatum* (ÖSTERGREN, 1947), *Festuca pratensis*, *Phleum phleoides* (BOSEMARK, 1954 and 1956 b) and *Zea mays* (RANDOLPH, 1941) (for a review see BOSEMARK, 1957). Another mechanism for numerical increase of the accessories has also recently been found in *Trillium grandiflorum* (RUTISHAUSER, 1956). ÖSTERGREN argues that such mechanism are sufficient to compensate for the meiotic loss of the accessories as well as the assumed negative selection against plants with such chromosomes.

This theory of the accessory chromosomes occurring as a kind of parasite has been rejected by several authors (WHITE, 1954; BOSEMARK, 1957). However, as has been stressed by WHITE, the hypothesis may hold true for the situation in certain cultivated plants where the relation between the population and the environment is a relatively recent one.

Nevertheless, as the hypothesis is based on a well developed mechanism for numerical increase of the accessories it can scarcely apply to the accessory chromosomes in *C. scabiosa*, *Caltha palustris* (REESE, 1954), and *Anthurium magnificum*, *crytallinum* and *forgetii* (PFITZER, 1957), which lack such a mechanism. Instead, it seems more likely that plants with accessory chromosomes have a positive selective value at least under some conditions. The idea that accessory chromosomes are useful has been put forward by many authors *e. g.* WHITE (1954) and DARLINGTON (1956). But it is naturally not meant that the effect of accessory chromosomes is unrelated to their number. If this were the case, the number of accessories would be capable of an unlimited increase. There is according to DARLINGTON an optimal number being different for different populations. Such an optimal number must, however, be relatively low as plants with higher numbers of accessory chromosomes occur relatively seldom in natural populations.

A similar opinion is held by WHITE (1954), who considers the accessories to be adaptive for the natural populations in which they have developed. He also points out that this adaptation must be rather delicately balanced and is liable to be disturbed in cultivated strains or under artificial conditions. These speculations are of interest in connexion with the situation in *C. scabiosa* where there is an indication that accessory chromosomes are especially well adapted under conditions which are considered more optimal or natural for the species.

An interesting suggestion as to the role played by the accessory chro-

mosomes has recently been made by DARLINGTON (1956). He considers that the accessory chromosomes may boost the variability and thereby the adaptability of a species. From this idea it is assumed that accessory chromosomes are especially useful for a diploid (where accessory chromosomes seem to be more frequent) and especially for a diploid which is forced to inbreed, experimentally or artificially. Thus, accessory chromosomes should be of especial value at the edge of the distribution range of a species where the populations are generally small. On the contrary, from the data obtained in *C. scabiosa* as well as in *Festuca pratensis*, it seems as if the frequency of accessories is often high where the species has its most common occurrence.

SUMMARY

(1) The geographical distribution of accessory chromosomes in *Centaurea scabiosa* was studied. Mainly natural populations in Scandinavia and Finland were investigated but collections were also undertaken from certain parts of England, France, Belgium—Luxemburg, Germany, Austria and Yugoslavia.

(2) The accessory chromosomes were present in all the investigated areas but in different frequencies. The accessory chromosome generally found is the standard type (FRÖST, 1956) but the accessory iso-chromosome, though occurring in a very low frequency, was also widely distributed.

(3) In Scandinavia and Finland there was a marked east-western difference in frequencies of accessory chromosomes. High frequencies were generally found in the southern interior of Finland and south-eastern parts of Sweden; and low frequencies were obtained in Norway, in the western parts of Sweden and in Denmark.

(4) In Sweden an agreement was obtained between humidity and frequency of accessory chromosomes. This relation seemed to hold true also for Norway and Denmark. The conditions in Finland seemed to constitute an exception to this rule.

(5) The continental climate in Finland as well as the continental features of the climate in eastern Sweden were contrasted with the more atlantic climate of the western areas.

(6) The average ecological differences between the eastern and western areas in Scandinavia and Finland were taken as an indication that plants with and without accessory chromosomes have different selective values under different environmental conditions.

(7) The significance of selective forces was discussed, and the possibility that the differences were mainly due to historical factors or to mere chance were also considered. No definite conclusion was drawn but it was assumed to be most probable that selective forces played the greatest role.

(8) The frequency of accessory chromosomes in different parts of Europe outside Scandinavia and Finland were, except in Austria, relatively low. Noticeable was the rare occurrence of accessory chromosomes in the Alps.

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STUDIES OF THE GENETICAL EFFECTS OF ACCESSORY CHROMOSOMES IN CENTAUREA SCABIOSA

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(Received December 3rd, 1957)

THe cytological behaviour, the inheritance, and the geograpical distribution of accessory chromosomes in *C. scabiosa* have been discussed in previous papers (FRÖST, 1956, 1957, 1958). This report will present some results concerning the influence of accessory chromosomes on fertility, speed of germination and vegetative development.

The material and methods used in these studies will appear in the text in connection with the presentation of the results.

I. OBSERVATIONS

1. Fertility

The material consisted partly of progenies after pair crosses (Tables 1 and 2), partly of progenies after open pollination (Table 3). Only pollen fertility was measured. The pollen was mounted in lacto-phenol-fuchsin and then classified. Table 1 gives the pollen fertility of progenies after a reciprocal cross of the type (20+1 acc. chr.) × (20+1 acc. chr.). As no difference between the two reciprocal progenies was obtained only the results of the summarized data are given.

It is fairly evident from the results that the presence of one and two accessory chromosomes do not affect pollen fertility in any measurable degree.

The pollen fertility was also determined in a progeny of a cross of the type 20 × (20+3 acc. chr.) but as about 40 per cent of the progeny-plants were semi-sterile the results are not presented here. The high sterility obtained in certain plants is probably not related to the presence or absence of accessory chromosomes.

In another experiment progeny plants from a single cross of the type 20 × (20+6 acc. chr.) were studied (Table 2). The number of plants

TABLE 1. *Pollen fertility in progeny plants from one reciprocal cross.*

Number of acc. chr.	Percentage of good pollen 50	60	70	80	90	100	Number of plants	$\bar{X}$ %
0			2	11	40		53	92.2
1	1		4	13	44		62	91.0
2		1		7	26		34	92.1
3					4		4	(95.0)
Total							153	

TABLE 2. *Pollen fertility in progeny plants from one single cross.*

Number of acc. chr.	Percentage of good pollen 30	40	50	60	70	80	90	100	Number of plants	$\bar{X}$ %
0							1	8	9	93.9
1							1	4	5	93.0
2					1	1		12	14	91.4
3	1					2	3	26	32	90.9
4					1	1	4	21	27	91.7
5							4	6	10	91.0
6							1	1	2	(90.0)
Total									99	

TABLE 3. *Correlation between number of accessory chromosomes and pollen fertility.*

Number of acc. chr.	Percentage of good pollen 30	40	50	60	70	80	90	100	Number of plants	$\bar{X}$ %
3—4							2	7	9	92.8
5—6				1		2	3	9	15	87.7
7—8			1				1	7	9	88.3
9—10					1	7	13	9	30	85.0
11—12				2	4	15	9	8	38	79.5
13—14			2		3	9	13	7	34	80.3
15—16				1	3	10	8	6	28	80.4
17—18		1		4	4	9	6	1	25	71.8
19—20				1	4	5	3	1	14	74.3
21—22						2			2	(75.0)
Total									204	

within the different categories is, however, rather small, considering the wide range of the chromosome numbers. From the results it seems that there is a small decrease of pollen fertility when the number of accessory chromosomes increases. However, the correlation coefficient is insignificant. Thus the influence of the accessory chromosomes on pollen fertility (in plants with up to 4 or 5 accessory chromosomes) seems to be rather weak.

From one natural population of *C. scabiosa* from the island of Gotland several plants were found with high numbers of accessory chromosomes. These plants were allowed to intercross freely and the seeds were mixed and sown. The distribution of chromosome numbers in these progenies was very wide and the material was thus used to determine the influence of higher numbers of accessories on pollen fertility. The results are given in Table 3. The different categories of chromosome numbers contain two numbers in each group since the determination of the exact chromosome number is rather difficult when the number of accessories is very high with several plants having been classified as 20 ± 14 acc. chr. and so on. A further reason is that the number of plants within the different individual categories is rather small. However, the correlation coefficient between pollen fertility and number of accessory chromosomes was found to be negative and significant at the 2 per cent level. In judging this result it must, however, be pointed out that the material must be genetically rather heterogeneous. Nevertheless, in spite of this fact it is probable that plants with higher numbers of accessory chromosomes have on an average a decreased pollen fertility.

2. Rate of germination

The influence of accessory chromosomes on the germination process has been studied in several progenies after paired crosses as well as in progenies after open pollination. The seeds were sown in petri-dishes, which were placed in thermostats. In general, three samples were taken out from the seed collection. After germination the first seedling root was fixed. Fixations were, however, undertaken at intervals of about every 24 hours.

However, as no significant influence of the accessory chromosomes on the germination capacity was obtained in any of the experiments no results are presented here. But in some trials done at higher temperatures ($+33°$), a weak indication was obtained that the seedlings

with higher numbers of accessories germinated more slowly than seedlings with lower numbers. It seems desirable to repeat these experiments but, in any case, it was evident from these experiments that the influence of accessory chromosomes on the germination process must be very slight.

3. Vegetative development

For the study of the influence of accessory chromosomes on vegetative development experiments were carried out in the field as well as in the greenhouse.

The effect of higher numbers of accessory chromosomes was determined in progeny plants. From two crosses of the types $20\times(20+7)$ and $20\times(20+8$ acc. chr.). The seed material was sown in small pots in the autumn of 1952. After fixation the plants were kept in a cold frame during the winter and planted out in the field during the spring of 1953. The plants were cut down in the autumn of 1953 and weighed individually excluding the root-systems. As *C. scabiosa* is a perennial species, the same procedure could also be repeated the following year. The results are given in Table 4. The correlation between plant weight and number of accessory chromosomes was calculated and the correlation coefficients were for both progenies, at the first as well as the second harvest, found to be negative. Only the coefficient for the cross K/57 1954 was significant at the 5 per cent level. Since the correlation coefficients were always negative, this must indicate that higher numbers of accessories have a negative influence on vegetative development.

Also the number of flowering heads (buds, flowers, ripe heads) was counted for each plant (Table 5). These measurements were done only during the year 1954. The correlation between number of flowering heads and number of accessory chromosomes was calculated and in both the progenies they were found to be negative but insignificant. Yet, in spite of the insignificance the negative coefficients may be taken as a further indication of a negative effect of high numbers of accessory chromosomes on vegetative development.

Experiments with low numbers of accessory chromosomes were described earlier (FRÖST, 1954) and strongly indicated that low numbers of accessory chromosomes have a stimulating effect on the vegetative development. To study further the effect of low numbers, an experiment was undertaken during the year 1957.

The material was derived from sister-plants and was divided into two

TABLE 4. *Correlation between number of accessory chromosomes and plant weight (grams).*

Number of acc. chr.	Average plant weight 1953	Number of plants	Average plant weight 1954	Number of plants
K/56 0	562.5000 ± 74.2500	8	1462.5000 ± 204.8000	8
1	750.0000 ± 70.7200	4	2125.0000 ± 295.5000	4
2	573.5294 ± 59.1200	17[1]	1465.7894 ± 151.5000	19
3	485.2941 ± 55.5400	17	1126.4706 ± 117.7000	17
4	536.6667 ± 45.9200	30	1326.6667 ± 92.2000	30
5	545.4545 ± 49.0100	22	1331.8182 ± 104.8000	22
6	491.6667 ± 52.8900	12	1133.3333 ± 89.4800	12
7	492.8571 ± 78.2600	7	1164.2858 ± 124.3000	7
Total		117		119
K/57 0	486.3636 ± 36.3700	11	1359.0909 ± 170.8000	11
1	450.0000 ± 47.6800	12	1350.0000 ± 98.4800	12
2	502.1739 ± 38.6900	23	1471.7391 ± 91.3100	23
3	450.0000 ± 32.3800	32	1243.7500 ± 83.5200	32
4	441.3043 ± 32.6200	23	1272.7273 ± 133.5000	22[2]
5	375.0000 ± 41.1800	8	1225.0000 ± 138.6000	8
6	492.8571 ± 77.5500	7	1150.0000 ± 138.1000	7
7	(550.0000)	1	(1750.0000)	1
8	(350.0000)	1	(1150.0000)	1
Total		118		117

[1] Two plants were used in crossing experiments.
[2] One plant was used in crossing experiments.

K/56 (1953) r = — 0.0883 insignificant
» (1954) r = — 0.1155 »
K/57 (1953) r = — 0.1164 »
» (1954) r = — 0.2093* $0.05 > P > 0.01$

groups, the one consisting of 105 plants all of which had no accessory chromosomes and the other of 143 plants all of which had 1 accessory chromosome. The two groups of sister-plants were planted out on the field, well isolated from each other. The plants within each group were freely intercrossed. From each of the two groups the harvest was undertaken so that two ripe heads were taken from every plant. The seeds were carefully mixed and sown out in green-house beds — 10 cm apart. Rows with different chromosome numbers alternated regularly so that rows of progeny plants after crosses between sister-plants without accessory chromosomes were growing between rows of progeny plants

TABLE 5. *Correlation between number of accessory chromosomes and number of flowering heads.*

Number of acc. chr.	K/56		K/57	
	Average number of flowering heads $\bar{X}$	Number of plants	Average number of flowering heads $\bar{X}$	Number of plants
0	137.5000	8	155.0000	11
1	135.0000	4	160.0000	12
2	175.5263	19	163.6957	23
3	129.7059	17	144.3570	32
4	146.6667	30	166.8182	22
5	146.8182	22	136.2500	8
6	128.3333	12	149.2857	7
7	130.7143	7	(255.0000)	(1)
8	—	—	(105.0000)	(1)
		119		117

K/56: r = — 0.0947 insignificant
K/57: r = — 0.0266 »

after crosses between sister-plants with 1 accessory chromosome. The experiment was planned in this way to avoid the time-consuming procedure of determining the chromosome number for every individual plant. For one category consisted only of plants without accessory chromosomes and the other, which was derived from crosses of the types (20+1 acc. chr.) × (20+1 acc. chr.), consisted of plants without, with one and with two accessory chromosomes in roughly the relation of 1 : 2 : 1 (but with an excess of plants without accessories (*cf.* FRÖST, 1957). From each of the two seed collections a sample of seeds was

TABLE 6. *Average plant weights in the experiment in the greenhouse, using progeny plants from intercrossed sister plants.*

Number of acc. chr.	Average plant weights (grams)		Number of plants
	I. harvest	II. harvest	
0	7.45	2.66	375
0.1 and 2 [1]	7.30	2.74	385

[1] The relation between chromosome numbers found in a random sample of 128 plants was: 43 per cent plants without, 42 per cent plants with 1, and 13 per cent plants with 2 acc. chr.

taken and germinated and the chromosome number within each category was determined (Table 6).

As the materials were derived from sister-plants it must be supposed that the average genotypical variation within the two categories is approximately the same. The materials differed therefore mainly as to their contents of accessory chromosomes.

The material was harvested twice, the first time three months after germination and the second time after another three months. The harvest was undertaken in the usual way, namely by cutting and weighing the plants excluding the root-system. The results are given in Table 6. At the first harvest the plant weight was slightly lower for plants with accessory chromosomes than for plants without. At the second harvest, on the other hand, plants with accessory chromosomes seemed to have overcome their slower start and the plant weight was slightly higher than for plants without accessory chromosomes. However, in neither the first nor the second harvest were the differences found to be significant when tested with the analysis of variance. The conclusion to be drawn from this experiment is that neither a negative nor a positive effect of a lower number of accessory chromosomes could be demonstrated with certainty.

Thus these results do not verify the earlier ones (FRÖST, 1954) where plants with 1 and 2 accessory chromosomes had on an average a higher plant weight than plants without accessory chromosomes, thereby indicating a stimulating effect of a low number of accessory chromosomes on the vegetative development. Some remarks must, however, be made with regard to the present material. The tested plants are the result of crosses between sister-plants, resulting in a certain degree of inbreeding. The effect of this inbreeding was manifested by a fairly high frequency of chlorophyll mutants among the progeny plants (about 10 per cent), which indicates a considerable number of sub-vital recessive genes in the material. A further indication was the occurrence of many plants with reduced vigour. The chlorophyll mutants were generally replaced by other plants whereas the other kinds of sub-vitals were not. However, the relative abundance of these recessive sub-vital genes probably results in a more discontinuous variation in the material which may conceal the eventual effects of accessory chromosomes.

II. DISCUSSION

Accessory chromosomes have been found in a large number of plants and animals and the knowledge about their behaviour and occurrence

is still increasing. Experimental data, however, on the genetical effects of such chromosomes within different species are still rare. Since it is impossible to decide by morphological characters if an individual has or has not accessory chromosomes, it was formerly assumed that they were genetically inert. Such a view was, for instance, supported by the conditions in maize where a plant could have very high numbers of accessory chromosomes without any visible effects being demonstrable. From the experimental investigations in maize (RANDOLPH, 1941) and in rye (MÜNTZING, 1943), however, it became evident that accessories could not be completely devoid of genetic activity. This fact was demonstrated by a decrease of fertility and vegetative development in plants with higher numbers of accessory chromosomes. The results in rye and maize and later also in other grass species (ÖSTERGREN, 1947, BOSEMARK, 1957) as to the effect of higher numbers of accessory chromosomes have also been verified in *Centaurea scabiosa*. A retarding effect was found on the vegetative development in plants with higher numbers of accessories but this negative effect was rather moderate. Pollen fertility also seems to be weakly affected although no negative influence was obtained in plants with up to 6 accessory chromosomes. In plants with still higher numbers of accessory chromosomes the decrease in pollen fertility was more pronounced. The influence on fertility thus seems to be less marked than, for instance, in rye where already low numbers of accessories gave noticeably negative effects. Also, accessories showed rather marked negative effects on vegetative development in rye plants with 6 to 8 accessory chromosomes. In maize, on the other hand, the number of accessory chromosomes can be increased to 15 or 20 before strong negative effects were obtained (RANDOLPH, 1941). The number of accessory chromosomes to cause detrimental effects can naturally be different for different species but probably varies even for different individuals within the species depending on their genotypical constitution as well as on environmental conditions. However, individuals with higher numbers of accessories are seldom found in natural populations. The most frequent numbers are the low ones which are therefore of special interest. Out of 2070 plants of *Centaurea scabiosa* with accessory chromosomes from 222 populations in Scandinavia and Finland about 73 per cent had only 1, 2 and 3 accessory chromosomes (FRÖST, 1958). There were variations in frequency of accessories between the different populations but the high percentage of these low numbers may indicate their dominance and importance for the plants.

The experiments undertaken in *C. scabiosa* and presented in this paper have not, however, given any evidence as to the effects of low numbers of accessory chromosomes. Fertility as well as speed of germination did not seem to be influenced by the presence of 1, 2 and 3 accessories. It was impossible to demonstrate any significant effects as to the influence of low numbers of accessories on vegetative development, neither in a positive nor negative direction. Nevertheless, in a previous experiment (FRÖST, 1954), evidence was obtained that low numbers of accessory chromosomes have a stimulating effect on vegetative growth. The results in the present case have been discussed earlier. It was assumed that the absence of any noticeable effect of the accessory chromosomes might depend on the occurrence of recessive sub-vital genes which caused a more discontinuous genetic variation in the material and might conceal the effect of the accessory chromosomes. As has been pointed out, a weak inbreeding had occurred and this can probably explain the segregation of a rather high percentage of chlorophyll mutants as well as other types of sub-vital plants. However, the effect of this inbreeding will also result in »a more homozygous state» within the material, which in itself may be less suitable for accessory chromosomes.

More extensive experiments to test the influence of low numbers of accessory chromosomes have mainly been undertaken in *Festuca pratensis* (BOSEMARK, 1957). From the results good indications were found of a stimulating effect of such chromosomes on the vegetative development. However, deviating results were also obtained so that no effect could be proved but in no case was any negative effect of low numbers found on the vegetative growth. The differences obtained between different materials were assumed to depend partly on differences in the genotypical constitutions of the material.

A different reaction of different plants containing accessory chromosomes is, however, what might be expected. This opinion is largely supported by the data obtained from the investigations of the geographical distribution of accessory chromosomes (*cf.* BOSEMARK, 1956 and FRÖST, 1958). These results show that in certain regions accessory chromosomes are very common and in others they are rare or practically absent. The differences in frequency of accessory chromosomes are probably not accidental but correlated with certain edaphic and climatic factors. It is impossible to decide whether the differences obtained in the experimental results are an expression of such assumed differences in reaction towards accessory chromosomes between differ-

ent genotypes or if they only demonstrate the difficulties of handling the experimental problems. Further experiments undertaken under fully controlled conditions and with material of different genotypical origin are certainly needed before the effect of accessory chromosomes can be fully understood.

SUMMARY

(1) The influence of accessory chromosomes in *Centaurea scabiosa* on fertility, rate of germination, earliness, and vegetative development was studied in various experiments.

(3) Fertility seems to be weakly affected by accessory chromosomes. No reduction in pollen fertility was obtained in plants with 1 and 2 accessory chromosomes and even in plants with up to 5 and 6 such chromosomes no significant decrease was obtained. In plants with higher numbers of accessory chromosomes the reduction in pollen fertility was found, however, to be more pronounced.

(3) No significant influence of various numbers of accessory chromosomes on the germination rate was observed.

(4) Strong indications were obtained that vegetative development was decreased by higher numbers of accessory chromosomes. In one experiment the effect of low numbers of accessory chromosomes was tested and no significant influence was obtained. This result does not agree with that obtained earlier where a low number of accessory chromosomes was found to give a stimulating effect on vegetative development. It was assumed that the material in the last experiment was less suitable because of inbreeding effects.

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ON THE GENETICS OF DWARF STRAINS IN PISUM

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(Received June 25th, 1957)

I. INTRODUCTION

DWARF strains are considered to arise in plants mainly in two ways. The one is due to degeneration of the plant's chlorophyll mechanism. Its most common cause is mutation in a gene, and this influence the chlorophyll synthesis. In the simplest case the morphological reaction is monohybrid segregation into normally green and chlorophyll-defective plants. Compare *e. g.* VON ROSEN, 1942 and 1954, chap. XVII, LAMPRECHT, 1952 and HOLM, 1954. Less frequently types emerge which are fitted for life up to the flowering stage and seed formation. In such cases they are semivital and are distinguished from normal plants not only by a lighter chlorophyll colour, but also by their dwarf growth (*e. g.* »chlorina» strains, RASMUSSON, 1938). In other morphological respects, however, such plants are quite normally developed. Their reduced assimilation impairs the plant's development.

The other way in which a genetically hereditary dwarf individual may be formed is by a mutation in one of the length genes, which govern the internodes and the extension of the plant. Temporarily a phenotypically similar dwarf plant may arise, of course, when its surroundings influence the food or water supply of the individual (*e. g.* low pH reaction in the soil, strong insolation *etc.*).

In *Pisum*, a number of length genes are known, which have been clarified and reviewed especially by RASMUSSON (1927), DE HAAN (1931) and LAMM (1938). It was discovered that, in addition to the fundamental gene for growth in length already described by MENDEL and designated *Le—le*, there exist a basic gene Cry_1 and a multiple allele series called Cry_2—cry_2^c—cry_2^s. Both *le* and cry_1 promote a dwarf-sized type of plant (named »dwarf»), whereas cry_2^c and cry_2^s effect the disappearance of the very dwarf type which the two previous genes brought into existence. With cry_2^c, the lower internodes of the stem

undergo abnormal growth in length (»crypto dwarf»). With cry_2^s, the great majority of the internodes are lengthened to an abnormally large extent (called »slender»). These dwarf phenotypes are described in detail in the publications quoted above. Moreover, WELLENSIEK (1946) gives an account of another kind of crypto-dwarf plants (called »springers») which in their genetical constitution probably form an intermediate combination, a »tall crypto dwarf». DE HAAN (1931) and RASMUSSON (1927) have studied similar combinations. LAMPRECHT (1954 b) describes a normal dwarf which also suffers changes in the formation of the floral and petaloid organs and is, therefore, sterile. RASMUSSON (1938) describes two new types of dwarf, the one semi-vital, called »Micro», and the other, lethal before seed formation, called »Ångström». LINDQVIST (1951) gives a detailed account of the genetics of the »Micro» dwarf and ascertains that a new gene *Lm—lm* produced this dwarf type. The great majority of these mutations in length thus occurred in the progenies following intra *Pisum sativum* crosses. (LAMPRECHT's sterile dwarfs are an exception, emerging from the cross *Pisum sativum*×*abyssinicum*, see below.)

A few dwarf mutants appeared in progenies of crosses between *Pisum sativum* lines and a strain from Abyssinia (*P. abyssinicum*, BRAUN) (*cf.* VON ROSEN, 1944). These cases must be regarded as representing true gene mutations, since they only appeared on one occasion and the respective types gave quite uniform offspring. Other data also strongly indicated true gene mutation and not simply segregation of existing genome constituents in this species cross.

II. RESULTS

During the winter of 1933—1934, different strains of *Pisum* were crossed by the author in the greenhouses of the Swedish Seed Association at Svalöf, owing to the kind co-operation of Prof. H. NILSSON-EHLE, head of the Association at that time. Two theoretical pea lines, obtained through Dr J. RASMUSSON from Dr DE HAAN in Holland (*cf.* DE HAAN, 1931), were crossed with the *abyssinicum* line; in the direction of the cross using *P de Haan 204* (Fig. 3 a) and *P de Haan 201*, respectively, as the mother, and *abyssinicum* as the father, there were obtained, among the very few pods received, three with very bad seed-setting (1 pod with 3 seeds and 2 pods with 1 seed from the same mother plant in the former cross, and 1 pod with 1 seed in the latter). The normal pods from this cross direction and the reciprocal cross produced

TABLE 1 a. *Analysis of the linkage situation in the mutated P de Haan 204-genome in F_2. Co %.*

Chromosome synonyms according to LAMPRECHT.

I	II	III	IV	V	VI	VII
D — Am			Le — V		P — Cry_2?	
36 (40. —)			16 (22.14)		17 (—. —)	

Am ——— 20: (—) ——— Gp

or

I — III

M ——— 30?! (50) ——— Gp

n = 70 — 150 plants

50 % Cross—over

Le — D Cry_2 — D D — V Am — V

» — Am » — Am » — M » — M

» — M » — M

Monohybrid segregations in 3:1	P for difference from 3:1
D—d	> 0.1
Pur—pur	< 0.001
Am—am	> 0.1
M—m	> 0.1
F—f	< 0.001
Le—le	> 0.1
V—v	0.01
Gp—gp	< 0.001
Pl—pl	< 0.001
Le—Cry_2	> 0.1 in 15:1
Fert—fert	> 0.1 in 1:1

the expected segregation results, whereas the offsprings of the pods discussed above showed very abnormal segregations.

The F_1 plots of the cross *P de Haan 204×abyssinicum* (nos. 35—38/1934) completely corresponded with the small mother line in character. The only divergency was shown by the pods which were coloured blue instead of green. The same relationships were seen in the F_1 from *P. de Haan 201×ab.* (plot no. 18/1934). Normally, in both cases, a tall and otherwise intermediate type would have been expected.

In 1935 an F_2 was sown from *P de Haan 204×ab.* Of the 150 seeds sown, 149 = 95 % germinated. This F_2 was cultivated on the plots 112 a—d/1935. The general appearance of the plants corresponded with *P de Haan 204* and not with *abyssinicum*. According to the field notes the following distinctive types were observed, *i. e.* 1/ tall, 2/ low, 3/ still smaller dwarfs and 4/ slender plants. See Figs. 3 a—e and Figs. 5 h—i. As to pod colour, green and blue segregated. There was probably segregation in flower colour (the faint pinkish white against the usual white colour, *i. e.* the gene *Am—am*) but this could not be decided with certainty. The colour of the seed coat *F—f* segregated (see LAMPRECHT, 1953 a). A closer analysis of the segregation ratios obtained in F_2 (Table 1 a) revealed extremely disturbed monohybrid segregations of the pod colours *Pur* and *Gp* (*cf.* LAMPRECHT, 1953 b). The divergences from 3 : 1 segregation of the *F* and *Pl* genes are probably connected with the hypostatic manifestation of these colour properties in the presence of the seedcoat colour gene *U* in *abyssinicum* (*cf.* VON ROSEN,

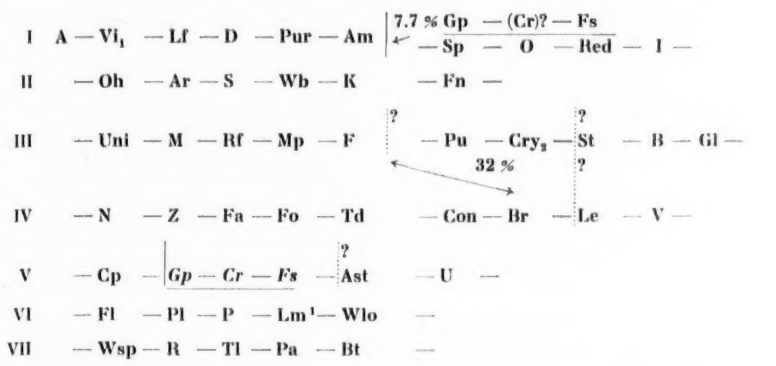
TABLE 1 b. *Analysis of the linkage situation in the mutated P de Haan 204-genome in F_2. Co %.*

Chromosome synonyms according to LAMPRECHT.

	I D	I Pur	I Am	II —	III M	III F	IV Le	V Gp	V Fs	VI Pl	VII	Monohybrid segregations in 3:1	P for difference from 3:1
I	D		50 (—.*40*)		50 (50.*50*)		50 (50.*50*)						
I	Pur		50!(20.—)		50 (45.*50*)		50 (—.*50*)	— (—.*50*)	— (—.*50*)	50 (—.*50*)		D—d	> 0.1
II	Am				50 (42.—)		50 (50.—)	7!(—.—)		50 (50.—)		Am—am	> 0.1
II	—											Pur—pur	> 0.1
III	M						50 (42.*50*)	38!(—.*50*)	— (—.*50*)	50 (50.*50*)		M—m	> 0.1
III	F						32!?(50.45)					F—f	< 0.001
IV	Le							50 (—.*50*)	— (—.*50*)	50 (50.*50*)		Le—le	> 0.1
V	Gp								— (—.*42*)	40?(—.—)		Gp—gp	> 0.1
VI	Pl											Pl—pl	> 0.1
VII	—												

n = 200 — 2000 plants

50 % cross—over
D — Am, D — M, D — Le, D — Pl, D — Cry_2
Pur — Pl
Am — Pl
M — Pl
Le — Pl


I A — Vi1 — Lf — D — Pur — Am
7.7 % Gp — (Cr)? — Fs
— Sp — O — Red — I —
II — Oh — Ar — S — Wb — K — Fn —
III — Uni — M — Rf — Mp — F
? — Pu — Cry2 — St — B — Gl —
32 %
?
IV — N — Z — Fa — Fo — Td — Con — Br — Le — V —
V — Cp — Gp — Cr — Fs — ? Ast — U —
VI — Fl — Pl — P — Lm1 — Wlo —
VII — Wsp — R — Tl — Pa — Bt —

[1] according to LINDQVIST, 1951.

Fig. 1. Gene map of the *Pisum sativum*-chromosomes according to LAMPRECHT.

1944 and LAMPRECHT, 1953 a). Table 1 a also reviews some new linkage phenomena that emerged from the few gene combinations which could be investigated, namely *Am—Gp* (co=20 %) and *M—Gp* (co=30 %). The latter linkage is doubtful, since LAMPRECHT in normal *sativum* located *M* in his chromosome III (see below) and *Gp* in chromosome V, whereas VON ROSEN in his species crosses placed *M* in chromosome I, whereas here too *Gp* is situated in the group corresponding to LAMPRECHT's Vth. The indication is, however, interesting since it would fit in well with the interpretation of the other F_2 and F_3 results which point directly to the existence of a translocation between chromosomes I and V. (See further below.)

Table 1 b, which follows, reviews the data from the segregating F_3 families. It can be seen that controlled monohybrid segregations are generally normal (for F, see above). But when the linkage values from *abyss.*×*sativum,* cited in parenthesis and taken from VON ROSEN, 1944, and intra *sativum* crosses (the latter values are bracketed and cursive) are compared with the latest *co* values obtained, the above-mentioned new linkage indications appear again, that is, *Am—Gp*

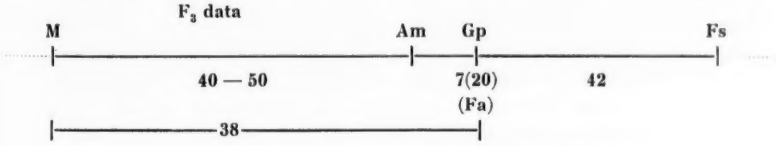


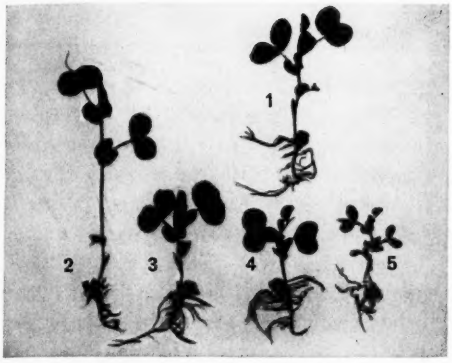
Fig. 2. Gene map of the translocated chromosome I.

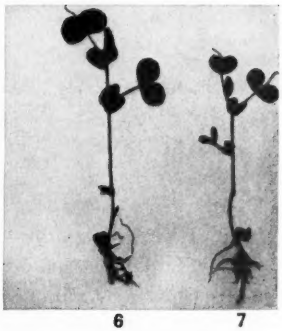
(7 %), *M—Gp* (38 %). The number of individuals behind each linkage calculation has varied between 200—2000, with 500—1000 as the most common number. The linkage values which are < 40 can, therefore, be generally regarded as statistically reliable. These new linkages transfer the gene *Gp* in the mutated line from chromosome V to chromosome I (see Fig. 1). A detailed picture of the appearance of the new translocated chromosome is reproduced in Fig. 2. An exchange of chromosome parts would thus have occurred between an outer (?) part of chromosome V and an outer (?) part of chromosome I. The chromosome ends in question *Gp—Fs* from no. V and *Am*—? in no. I are more likely to be found, according to LAMPRECHT's calculations, in the centre of the respective bodies. The problem of their exact positions cannot be further elucidated from our present knowledge alone. In this respect the important point is that a previously unknown translocation in *sativum* has been demonstrated in combination with the unexpected segregation of height genes in the progeny following *P de Haan 204* $\times$ *ab.* Possibly another demonstrated combination between chromosomes III and IV may be indicated by the linkage *F—Le* (co=32 %).

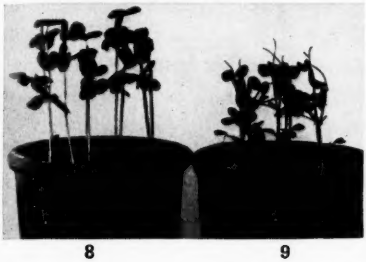
From the length classes obtained in F_2, pedigrees were drawn which were followed generation by generation as far as to F_8 and partly to F_{12}. They have continually proved to be constant and always completely fertile. A limited cytological analysis of the meiosis of these lines demonstrated fully normal divisions.

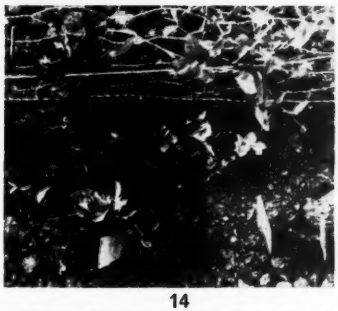
F_2 of the cross *P de Haan 201* $\times$ *ab.* was cultivated on plot no. 108/1935. The number of plants was small but a clear differentiation in some varying types of low height could be observed. All the plants were described as white in flower and waxless; this indicates the absence of the *abyssinicum* genome. It was not possible to carry out any gene or linkage analyses. From the cross offspring a divergent low dwarf-like type was classified which in a pedigree cultivation of 8—10 generations proved to be completely constant. Meiosis-control established the presence of amphibivalents; this corresponded with the semi-sterility ascertained in the pods. As is stated later, this mutation can, however, be considered as analogous with those occurring in the *P de Haan 204* genome.

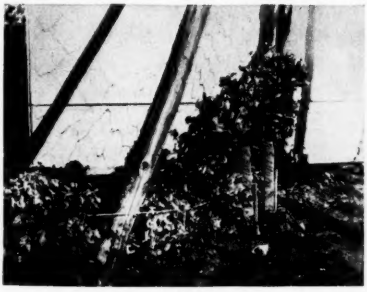
The cause of these mutations may possibly be due to some physiological disturbance which suppressed the product of the cross during the earliest embryonic development so that an abnormal, possibly pseudogamous, embryo development commenced. In combination with this, reciprocal translocations were probably provoked.











Figs. 1—13. Seedlings of mutated *Pisum*-dwarfs. Fig. 1, P de Haan 204; Fig. 2, mutant »Tall»; Fig. 3, mutant »Dwarf»; Fig. 4, mutant »Low dwarf», Fig. 5, mutant »nana»; Fig. 6, tall *c d w*?; Fig. 7, normal *c d w*; Fig. 8, tall *c d w*?; Fig. 9, normal *c d w*; Fig. 10, mutant »Dwarf»; Fig. 11, P de Haan 204; Fig. 12, mutant »Low dwarf»; Fig. 13, mutant »Nana». Figs. 14—16. Field plants. Fig. 14, mutant »Naby[32]»; Fig. 15, F_4: mutant »Low dwarf» and mutant »Dwarf»; Fig. 16, F_4: Tall and slender plants.

In order to keep the discussion of the genetical background and mapping of the translocations as brief as possible, the reader is directly referred for details of the questions involved to the comprehensive publications on the subject by LAMPRECHT, (1948, 1950, 1953 a and c, 1954 a and b) and also by VON ROSEN (1944).

In order to clarify the length factors of the new height lines the author carried out a large number of backcrosses with genetically known *sativum* lines. Unfortunately, the »tall» lines has not been investigated in the same way although the author is of the opinion, as other workers have previously pointed out, that it would be impossible to distinguish the expected »tall» medium types of crypto-dwarf in the segregation and assortment. (*Cf.* Figs. 4 f—g and 5 a—b with 4 b.) Free combination in the F_2 and F_3 linkage analysis of the mutated »tall» line between the gene *Le* and the genes *Am*, *M*, *Gp* and *Fs* indicates, however, that the mutation or translocation is not exactly the same in this case as in the new low types. No backcrossing with the slender type produced any seed.

In order to simplify the description of the new length classes, they were given special names. The new low line from *P de Haan 204*×*ab.*, which resembles the mother type but segregated in certain single genes, was designated *mutant* »Dwarf». (See Figs. 3 e, 4 c and 5 c.) The other height line of the same origin was called *mut.* »*Low dwarf*». In habitus it is similar to the normal type but is only half as tall, its lower internodes being arranged in a marked zig-zag order. (See Figs. 3 d, 4 d and 5 e and 6 b.) From *P de Haan 201* as mother line there emerged a very small dwarf type (Figs. 3 c, 4 e and 5 f), which does not zigzag so much in the lower internodes, but branches out in a marked fashion (like mut. »Low dwarf»). This very small dwarf type was called *mutant* »*Nana*». The backcrosses were made with the theoretically well-known *sativum* lines: *Grå dvärg* (*Gd*), *Witham Wonder* (*WW*), *Acacia* (*Ac*), *P de Haan 204* (*P d H 204*), *P de Haan 201* (*P de H 201*), Crypto-dwarf from RASMUSSON (*Cdw*) and *Slender de Haan* (*Sl*) (see gene characters in VON ROSEN, 1944, p. 266). The analysis of these backcrosses is given in Tables 2—4. Owing to the complicated nature of the analysis we shall not burden this article with a detailed account of the particulars and results of the various test crosses. Instead the tables are so detailed that they ought to furnish those especially interested in such *Pisum* analyses with the opportunity of judging for themselves the author's interpretation of the results. (The author wishes to state that individual separation of the low types in the assortment of the segregations was

TABLE 2. *F_2 analysis of the »Dwarf» mutant from the P de Haan 204 genome.*

Year	Sat.—line	Gene symbols			x	Mutant	Gene symbols			F_2 phenotypes Number of plants					Type of segregation						D/m	n
		Le	Cry_1	Cry_2			Le	Cry_1	Cry_2	dwarf	cdw	slender	»Low dwarf»	»Nana»	Le	Cry_1	$cry_2{}^c$	$cry_2{}^s$	$Cry_2{}^{lw}$	$Cry_2{}^{na}$		
1941	Gd	O	O	+		»Dwarf»	O	O	$+^{dw}$	296						hom		0!1/*16*			—	296
1942	»		»		x	»		»		1107						hom		0!1/*16*			—	1107
1942	WW	O	+	O^c	x	»	O	O	$+^{dw}$	485	32		35			15*d*	:1 ±				0.5	552
																15			:1—		0.5	
1941	Ac	O	+	O^s	x	»	O	O	$+^{dw}$	188		10				15*d*		:1—			0.8	198
1942	»		»		x	»		»		329		17				15*d*		:1—			1.2	346
1942	PdH_{204}	O	+	O^s	x	»	O	O	$+^{dw}$	581				9		63*d*		0!?**1/16**		:1	< 0.1	590
1941	Cdw	O	O	O^c	x	»	O	O	$+^{dw}$	145	35					3	:1-3/*16*	0!1/*16*			0.6	180
1942	»		»		x	»		»		549	110					3	:1-3/*16*	0!1/*16*			5.7	659
1942	Slender	O	O	O^s	x	»	O	O	$+^{dw}$	29		5				15		:1-*1/4*			1.4	34

TABLE 3. *F_2 analysis of the »low dwarf» mutant from P de Haan 204 genome.*

Year	Sat.—line	Gene symbols			x	Mutant	Gene symbols			F_2 phenotypes Number of plants					Type of segregation						D/m	n
		Le	Cry_1	Cry_2			Le	Cry_1	Cry_2	dwarf	cdw	slender	»Low-dwarf»	»Nana»	Le	Cry_1	$cry_2{}^c$	$cry_2{}^s$	$Cry_2{}^{lw}$	$Cry_2{}^{na}$		
1942	Mut					»Low																
	»Dwarf»	O	O	$+^{dw}$	x	dwarf»	O	O	$+^{lw}$	156			40			3			:1—		1.6	196
1942	Gd	O	O	+	x	»	O	O	$+^{lw}$	71			32			3		0!*1/16*	:1+		1.3	163
1943	»		»		x	»		»		569		29	91			15		:1-*1/16*			3.5	689
																3			:1—		3.8	
1941	WW	O	+	O^c	x	»	O	O	$+^{lw}$	37	1					15*d*	:1-**1/16**				1.4	38
1943	»		»		x	»		»		455			309			3*d*	0!?**1/16**		:1+		8.7	764
1943	Ac	O	+	O^s	x	»	O	O	$+^{lw}$	487		12	222			63*d*		:1-**1/16**			0.1	721
																3			:1+		3.4	
1943	PdH_{204}	O	+	O^s	x	»	O	O	$+^{lw}$	313			262			3*d*		0!?**1/16**	:1+		9.8	575
1943	Cdw	O	O	O^c	x	»	O	O	$+^{lw}$	self fertilized seeds							**3/16**	*1/16*				4
																3/4?	**1/4**?					

TABLE 4. *F_2 analysis of the »Nana» mutant from the P de Haan 201 genome.*

Year	Sat.—line	Gene symbols			x	Mutant	Gene symbols			F_2 phenotypes Number of plants					Type of segregation						D/m	n
		Le	Cry_1	Cry_2			Le	Cry_1	Cry_2^{na}	dwarf	cdw	slender	»Low dwarf»	»Nana»	Le	Cry_1	cry_2^c	cry_2^s	Cry_2^{lw}	Cry_2^{na}		
1942	Mut																					
	»Dwarf»	O	O	+	x	»Nana»	O	O	$+^{na}$	48						hom				0!?1/4	—	48
1942	Gd	O	O	+	x	»	O	O	$+^{na}$	104				26		3*d*				:1—	1.4	130
1943	WW	O	+	O^c	x	»	O	O	$+^{na}$	279	12		38	26		63	:1+*1/16*				1.8	355
																15				:1+	2.7	
																15				:1+	0.7	
1942	Ac	O	+	O^s	x	»	O	O	$+^{na}$	12		1		9		15		:1+*1/16*			0.4	22
																3				:1+	1.4	
1942	PdH_{204}	O	+	O^s	x	»	O	O	$+^{na}$	5				5		3		0!*1/16*		:1+	1.6	10
1943	Cdw	O	O	O^c	x	»	O	O	$+^{na}$	18	2					3	:1-*1/4*				2.2	20
—	»Low																					
	dwarf»	O	O	$+^{lw}$	x	»	O	O	$+^{na}$	No successful cross!									3/4?	:1/4?		0
—	PdH_{201}	O	O	+	x	»	O	O	$+^{na}$	No successful cross!						3/4?				:1/4?		0

sometimes doubtful, owing to local edaphic variations (*e. g.* late germination, variation in the earth conditions *etc.*). Such difficult classifications are marked in the tables. In the final collations, survey and analysis of the figures the author combined the reliable and doubtful groups (the latter of which always being in such a minority that they could not fundamentally influence the interpretations). The tables include the gene construction of the test-types, the frequencies discovered in F_2 between the crosses of various length classes and also the segregation scheme, with which the segregations discovered are considered to correspond (cry_2^c refers, as usual, to crypto-dwarf plants, cry_2^s = slender plants, Cry_2^{dw} = mut. »Dwarf», Cry_2^{lw} = mut. »Low dwarf» and Cry_2^{na}=mutant »Nana»). The segregation schemes to be expected from a normal *sativum* cross between known gene characters is indicated by cursivation. Here *d* means normal dwarf. The table also shows the certainty of the difference by which the discovered segregation ratio diverges from the normal. It can be observed in the scheme that the slender plants expected in several crosses do not appear. The »Nana» plants which occurred in the *P de Haan 204* cross are for the present impossible to explain, since typical »Nana» plants really belong to the offspring of *P de Haan 201*.

The results of the backcrosses indicate a gene constitution for the *mutant »Dwarf»* according to the *formula*: *le* Cry_1 Cry_2^{dw} (Table 2). The analysis of mutant »Low dwarf» is given in Table 3. As can be seen, the segregation occurs above all in normal dwarf and »Low dwarf» individuals. Here too the expected slender plants are missing and in one case crypto-dwarf plants also. It will be observed that the two series of crosses with *Witham Wonder* (*WW*) produced somewhat varying results (the number of individuals was very slight in 1941), and also that the departure from the 3:1 scheme in the backcross with *P de Haan 204* resulted in a D/m of 9.8: in both cases these divergencies may be connected with difficulties in classifying the plants in the field. The result of the F_2 analysis of these tests of mut. *»Low dwarf»* produced a *gene formula* of: *le* Cry_1 Cry_2^{lw}.

Finally, Table 4 reviews the results of the test crosses with mut. »Nana» from *P de Haan 201*$\times$*ab*. The backcrosses revealed disturbing sterility, which decimated the number of plants in the progeny; this was presumably caused by the »Nana» type's own sterility. The analysis is, therefore, quite uncertain. Here again examples of slender plant loss are to be noticed. It is characteristic that the main part of the segregations produces only individuals of the dwarf and »Nana» type.

The lack of »Nana» plants in the cross between mut. »Dwarf»×mut. »Nana» is curious. The result indicates epistasis and supports the opinion of the author that all the three mutations occurred in the same locus. The analyses state that the *mut. »Nana» gene formula* is *le* Cry_1 Cry_2^{na}. (Note that F_1 manifests the dominant characters of the parents everywhere in the backcrosses.)

III. DISCUSSION

A comparison of the gene formulas of the various original lines and their mutant forms is given in the following table where for the sake of simplicity the dominant gene is marked with + and the recessive gene with o. The arrows signify that the gene in question has mutated. The heading gives the various multiple alleles which are included in the respective series:

	Le	Cry_1	Cry_2	*A*	*Am*	*Wb*
P de Haan 204	o	+	o^s	+	o	+
Mut. »Dwarf»	o	o↓	$+^{dw}$↓	+	o	+
Mut. »Low dwarf»	o	o↓	$+^{lw}$↓	+	o	+
P de Haan 201	o	o	+	o	+	o
Mut. »Nana»	o	o	$+^{na}$↓	o	+	o

From this table it can be seen that the mutants from *P de Haan 204* mutated in two loci. Cry_1 passed to recessive and Cry_2 changed to dominant. The mut. »Nana» should only have changed in Cry_2 from the dominant normal locus to another allele of the dominant gene. All three mutant types should together form a new, multiple allele series, previously unknown in *Pisum*, of a dominant character. (*Cf.* LAMPRECHT, 1953 b, for other known allele series in *Pisum*.) Dominant allele series in *Pisum* are not exactly new; series *D—Dw—d* has been known for a long time for the colouring of the maculum ring, (see *e. g.* LAMPRECHT, 1953 a). The deficiency of slender individuals, the absence of linkage tendencies with the *Le* gene in chromosome III (according to LAMPRECHT's scheme), and the assigning of breakage points to chromosome I and V with a reciprocal interchange indicate together that it is precisely the third basic gene for length factors which would participate in this mutation. Cry_2 has not yet actually been placed on the chromosome map, but there are indications that it may lie close to *Am* in chromosome I or *Gp* in chromosome V. With reference to LINDQVIST's (1951) careful analyses of the *Lm* gene of mutant »Micro», it is clear

that this gene cannot be suspected of participating in this alteration of dominance. Evidence of a certain special linkage between chromosomes III and IV (*Le—F*) indicates that Cry_1 in some way took part in the change but, if so, in the normal change from dominant to recessive. The change in Cry_2, however, occurred in the less usual direction of recessive to dominant. The author considers that this dominance and the result of the linkage analysis show that the new mutant types are conditioned not only by a single gene mutation in *one* genetical locus, but that the change in position occurring in connection with translocation also promoted examples of new position effects of the real chemical gene (*e. g.* an aminoacid on the nucleoproteid chain). Thus various dominant alleles in the series Cry_2—Cry_2^{dw}—Cry_2^{lw}—Cry_2^{na} may be specific effects of some aminoacids and other types of basic gene materials in respect of their different reactions in the pea plant. This dominant series is, therefore, all the more interesting; it offers a correspondence with the recessive multiple series demonstrated earlier, namely Cry_2—cry_2^c—cry_2^s. In comparing both series one may venture to assume that the position effects of *e. g.* aminoradicals in the dominant series coincide in some way with those in the recessive series. Bearing in mind that research now considers that the gene is mainly constructed of nucleic acids, the author would like to put forward the working hypothesis that *e. g.* an amino acid chain or similar salt-forming compound burst free and that in combination with a simultaneously occurring chromosomal translocation some keto or hydroxyl group paired together and thus, through this chemical process, the reaction system of the whole gene was changed. Chemical systems of growth prohibition could thus arise, with dwarf plants as a phenotypical manifestation. But the whole molecular complex should constantly function from the genetical point of view as one collective gene locus. It is the task of future research to interpret the chemical background of such a series of mutations as is reported here.

Besides the dwarf types of *Pisum* analysed above VON ROSEN (1954, p. 217) published an account of a dwarf form in which, after some weeks, the prohibition effect disappeared and normal growth began. It arose after treating *P. abyssinicum* with the phosphorous isotope P^{32}. This strange dwarf type is named »*Naby*^{32}» (=»Nana» type in *abyssinicum* after treatment with P^{32}).

Chapter XVII of VON ROSEN (1954) describes a back mutation of the maculum gene $d \rightarrow D$, which arose with high frequency following treatment both with phosphorous isotope and with mustard gas. The

cause may be breakage in an older, translocated locus, $D \rightarrow d$ (VON ROSEN, 1944) which has long been well known. The small dwarf plants which arose in the great majority of X_4 families in 1955 may have originated in a similar way (see Fig. 4 g). The families segregate irregularly in generally small numbers of individuals. The *D*- and *d*-alleles were just as often represented. Sterility was general. An estimation of sterility, judged by the seed-setting of the pods of the whole material, resulted in the following correlation between the *D*-factor and semi-sterility: *D* and fertile=33 plants, *D* and semi-sterile=297, *d* and fertile=41 and *d* and semi-sterile=242 plants. The translocation is, therefore, directly connected with semi-sterility.

In the X_4 generation of this material, fixations of *D*- and *d*-plants were made separately. Many hundreds of buds were fixed, but as is usual with the *abyssinicum* line only a small numbers of buds were obtained with meiotic divisions suitable for cytological analysis. A contributory cause to the unusually poor result may have been the abnormally high temperature and slight rainfall prevailing in the summer of 1955. The analysis indicates, however, that there is a higher frequency of plants with disturbances in A_I and A_{II} (bridges, fragments, *etc.*) in *D*-individuals by comparison with *d*-individuals. In both types of plant M_I and M_{II} were practically normal. Moreover, the tetrads in *D*-plants exhibited micronuclei more often than those in *d*-plants. This indicates that *D*-individuals from the maculum mutation lines contain a chromosome rearrangement differing from the *abyssinicum* line, whereas the *d*-plants, originating from *D*-plants, arose through a back rearrangement of the altered chromosomes in *D*-individuals. Here the colourless maculum ring typical of the *abyssinicum* line manifested itself again. The *d*-individuals have not recovered a normal *abyssinicum* genome, however, since they still exhibit individuals with disturbed meiosis although with less frequency than the mutated *D*-lines.

From this deviating gene background dwarf individuals later developed in the X_4 generation through some defect in plant development. The progeny of these X_4 dwarfs were checked in X_5 1956. Out of 90 tested X_5 families, 84 were found to give pure dwarf individuals. The remaining six families were normally tall and probably originated from phenotypically »low» X_4 plants which, owing to weak development, have been misjudged. 7 plants normally tall in X_4 segregated in X_5 into tall and dwarf plants. Altogether 79 tall to 26 dwarfs were counted. The ratio corresponds to an ideal 3 : 1 segregation with $D/m = > 0.1$. A normal *abyssinicum* population has an average height of 50 cm. Amongst

the dwarf families in X_5 two dwarf types, each with its own distinctive height, could be discerned, namely, one low with an average height of 20 cm, and a medium low with a height of 35 cm. The presence of two separate dwarf types in this material recalls similar conditions following the cross *P de Haan 204*×*abyssinicum*, described above. The germination energy in X_4 was at least as good in the dwarf plants as in the normal plants. The number of seeds per pod was also equivalent to that of the normal plants.

	D-norm	*D*-dwarf	*d*-norm	*d*-dwarf
$\bar{x}$ seeds/pod in X_4	2.1	2.5	2.8	2.2
% germinated seeds in X_5	42.3	64.7	50.7	59.8

The dwarfs, therefore, seem to be almost more fertile than the normally tall plants. On comparing *D* and *d* plants no difference appeared, however, in seeds per pod or in the germination (on an average 52.7 against 53.5 %, respectively). Single plants defective in chlorophyll content arose in X_5, although they were now less numerous than in previous generations.

From about 180 X_4 offspring, tested in X_5, belonging to »mutated red maculum plants», there were 16 families which segregated, originating from X_4 *D*-plants. The segregation figures were 128 *D* : 39 *d*, which corresponds to a normal 3 : 1 ratio (D/m=0.5). Equivalent data for 4 *d*-families amounted to 20 *D* : 8 *d* (with 3 : 1 D/m=0.4).

In all, 178 X_4 plants were evaluated by their progenies in X_5. Of these, 79 plants in X_4 were classified as *D* and 99 as *d*. The evaluation of the maculum colour in X_5 produced the following relationships between the maculum gene's manifestation in X_4 and X_5. Out of the 79 *D*-plants in X_4, 56 families were homozygous *D* in X_5, 16 families segregated in *D*—*d* plants, and 7 families were homozygous for *d*. The offspring of 99 X_4 *d*-plants comprised 91 homozygous *d*-families, 5 *D*—*d* segregating families and 3 constant *D*-families. Even considering the possibility that some plants may have been incorrectly evaluated both in X_4 and X_5 and that the number in certain families was low, these data nevertheless indicate that the maculum gene in these lines is to be regarded as a »labile» gene, since the corresponding phenotype was changed in 3—10 % of the pedigrees studied.

In the maculum mutant offspring cultivated in X_5 there also appeared families in which either all the plants or only some plants were decidedly taller, more robust and later developed than the normal type. Single plants like these have already been observed in previous gener-

ations (VON ROSEN, 1954, p. 219). Normally, as already mentioned above, *abyssinicum* is about 50 cm high and the average date for flowering occurs in the middle of July. The deviating plants had an average height of 65 cm and an average flowering date which was at least two weeks later. 18 out of 100 families were found to be homogenously late. Two further families contained single late plants. The size of the flower, measured on the width of the wing, is in the mother type usually 1 cm or less. In the offspring of X_4-*D*-plants, 74 out of 84 families possessed flowers of normal size, one family had the wings 1—2 cm, and 9 families $\geqq 2$ cm wide. In the offspring of X_4-*d* plants, 81 out of 93 families retained the normal flower size, 2 had an intermediate type, and 10 had large flowers. In contrast to what the author believed in his work of 1954, p. 219, these data on X_5 do not point to any close connection between the maculum gene mutation and the increase in the size of the flower.

Nor is it probable that the predisposition to the number of folioles directly linked with the maculum gene change. A normal *abyssinicum* plant possesses only *one* pair of developed folioles per leaf-stalk. In X_4 and X_5 quite a large number of plants have been observed with one—two or regularly two pairs of folioles. 84 progenies of *D*-plants comprised 38 families with one pair of folioles, 37 intermediates, and 9 with two pairs. 93 progenies of *d*-plants comprised 61 normal families, 30 intermediates, and 2 families with two pairs.

The comparison between X_4-plants and their offspring in X_5 thus shows that the maculum gene in these mutated lines is not completely stable and that the segregation into late plants of greater height, larger flowers or an increased number of folioles is not necessarily due to the maculum gene.

In origin and probably also in structure the typical dwarf form described above can with some certainty be compared with the mutations of the *P de Haan* lines previously described. It is called »Naby», since it is a »Nana» form from *abyssinicum*.

Quite a different type of altered morphology in the plant is exhibited by the variations usually designated as »rouge» plants. A more detailed description of this phenomenon and other similar cases is given in VON ROSEN, 1944, pp. 353—354. These arose as isolated individuals in the progeny of the cross *Pisum sativum*×*abyssinicum* and they are constantly morphologically deformed and sterile. (*Cf.* LAMPRECHT's mutated dwarfs (1954 b). The author also observed several individuals with this appearance amongst plants growing in earth-filled pots to

which are added various large amounts of complex-forming metal ions as also among the descendants from neutrone-treated seeds (VON ROSEN, 1957). These abnormal individuals were always sterile. The background to the emergence of the type could justifiably be defined as misdirected enzyme production.

DE HAAN and GORTER (1936) made a similar suggestion with regard to the »slender» plants. Using varying methods of analysis differences were found in the auxin and catalase content between slender plants and normal tall plants. The auxin reappeared in stronger concentrations in the long lower internodes while at the same time an obvious catalase inactivation of the auxin occurred. On the other hand a higher catalase content was found in the tops of normal tall plants; here the breakdown of the auxin was larger so that growth was stopped. The length of the internode as between slender and tall plants was determined at 3.8 : 1, the number of cells at 1.4: 1, the total length at 2.3 : 1, and the amount of catalase about 1: 2.1. Slender plants should thus contain about twice as much auxin as the corresponding tall pea line. For further information on the problem of the presence of auxin in the living cell tissue it may be said that HEMBERG (1954) stated that maize seeds when steeped in auxin solution easily absorb it and increase the concentration of free auxin more than five times, in contrast to that bound inactive in the tissue. After 24 hours the content of bound auxin was, on the other hand, double that of free auxin; a change had occurred from a free to a bound form. Again, after steeping in water alone little free auxin was found but much bound auxin. After 24 hours the opposite was the case. These preliminary tests probably show that auxin (in this case indolyl acetic acid) is converted very rapidly in the tissues and is dependent on other enzymatic constituents of the cell tissue, *e. g.* inactivating catalases.

If, as in the ordinate in Fig. 6, the various known length classes in *Pisum* are distributed according to the height values characteristic of the lines, and along the absciss the hypothetical values of the auxin and catalase content are set, based on knowledge gained from DE HAAN and GORTER's results, then one obtains a successive increase of auxin content together with a rising series of length classes and diminishing catalase content. In the recessive allele series the active quantity of auxin should be increased, while the growth in length is accelerated. Possibly this happens owing to the decrease in the quantity of inactivating catalase. Conversely, in the dominant allele series the quantity of auxin should diminish and thus growth stops. This is effected by

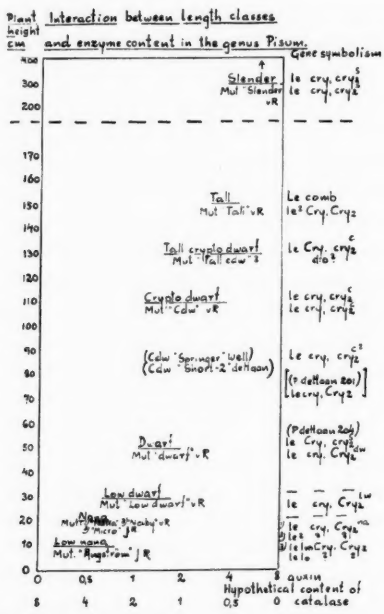


Fig. 6. Interaction between length classes and enzyme content in *Pisum*.

increasing the catalase content so that the greater part of the auxin present is inactivated. On the right of the figure proved or assumed gene formulas are given for the length classes concerned, in accordance with the previous account. A connexion can be observed between the effect of the auxin content on the length reaction and the Cry_2 factor's change in its two allele series (the dominant and recessive series), secondly, with the Cry_1 factor also. For long scientists have sought to explain in *Pisum* the cause of the acceleration in length amongst crypto-dwarf and slender types, suggesting that it was due to a cessation of function in a growth-prohibiting gene. The interpretation of the diagram reproduced here should, also, the author considers, lead one to exactly this explanation. The dwarfs, in particular the extreme forms »Nana», »Micro» and »Ångström», would then instead be due to an accentuation of this growth-prohibiting function, *e. g.* through increased catalase content. LUNDEGÅRDH (1950) states that auxin is thought to function with narrow optimum margins so that even an insignificant

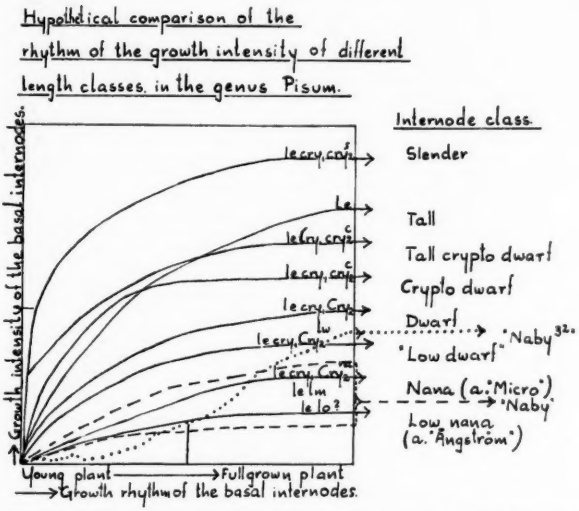


Fig. 7. Hypothetical comparison of growth curves of different length classes in *Pisum*.

change in concentration can have a great effect. It is kown that nana forms may emerge through the excess of some kind of enzyme.

Whether the above hypothesis can be experimentally proved will be seen from future research. Should this hypothesis really be confirmed then other known series of multiple alleles could be included by a similar explanation of such principles, *e. g.* the known *Pal*-series in *Antirrhinum*, the flower series in *Pharbitis*, the seed colours in maize and parallel series in animals, *e. g.* the colour series of rabbit fur, the pigment series in butterflies, vestigial and eye colour series in *Drosophila* and so on. With regard to the latter, GOLDSCHMIDT, (1938) has presented his theory concerning the progressive reactions of the enzymes during the separate periods of the various embryonic stages of the fly larva (*e. g.* p. 59 of the above publication). If the same approach is adopted, with GOLDSCHMIDT's theory as starting point, for the reactions of the length factors in the pea plant, one can also assume the presence of a series of various gene-governed points of concentration, and of one or more growth enzymes which accelerate growth at different rates in the separate developmental phases of the plant individual.

A further development of this trend of thought is presented in Fig. 7.

The ordinate shows here the intensity of growth in *basal internodes*, and the absciss the rate of growth. Every known length class is given its hypothetical curve of growth based on its known gene constitution, as stated above. From this it appears that the gene symbols, *le* Cry_1 and Cry_2 with their various prefixes, promote enzymes which prohibit growth in length. The opposite gene symbols lack effect or accelerate this. Note the rapid initial growth of the slender types. Crypto-dwarf is also accelerated in the beginning. Dwarfs, and especially the extreme dwarf types, exhibit only a retarded growth rate. Normal tall pea grows at a fairly quick and even pace into the adult, flowering plant. On the other hand, the curious dwarf mutant »Naby^{32}», arising from treatment with phosphorous isotope displays quite a different curve: first slow and then quickening somewhat.

When the functioning of the gene is traced back to enzyme reactions, in GOLDSCHMIDT's sense, a working hypothesis is obtained to explain such a special case as the length factors in *Pisum*. The linkage functions of the gene system and its special reactions, together with the emergence of new reaction norms connected with changed specific chemical effects of the genes, should thus function so that the strength of the growth reactions should progressively increase with the change in the gene constitution. The enzyme reactions described correspond, as far as their morphological manifestations are concerned, in more than one respect to the growth-promoting effects demonstrated with *e. g.* the elements Mg, Ca, Zn, Al and Mn (VON ROSEN, 1957). Support for such a relationship is also provided by the information that the metals Mn, Co and Ni activate the enzyme arginase, that Zn inactivates auxins, and further that catalase belongs to the growth-prohibiting enzymes which contain iron (LUNDEGÅRDH, 1950). BURSTRÖM and TULLIN, 1957, likewise remind us that Fe, Mg and Co are fixed in porphyrins, Mg, Mn and Zn to proteins as enzyme activators and Cu in oxidaces. The metal ions are bound in chelate formations to proteins. The balance in the cell-system between different chelates also seems to be very important for a normal cell metabolism. Recently the author has been successful in establishing the fact that simple metal ions such as Cu^{++}, Zn^{++}, Mn^{++}, Fe^{++} *etc.* are able to induce real gene-mutations in peas: *e. g.* different types of chlorophyll-deficient or waxless individuals (VON ROSEN, 1957). Thus one is probably justified in assuming that there are connections between metal ions and the phenomena discussed in this article.

SUMMARY

An account is given of known dwarf types in *Pisum*, and a number of dwarf mutations are described. A new dominant allele series in the crypto-dwarf factor Cry_2 is demonstrated: Cry_2—Cry_2^{dw}—Cry_2^{lw}—Cry_2^{na}. This gene series, which produces dwarf types, should correspond with the previously known recessive allele series Cry_2—cry_2^c—cry_2^s, which, on the contrary, promotes increased growth in length. Tests were made in order to combine all the mutants in the length factors under a common auxin reaction system on a par with GOLDSCHMIDT's vestigial-wing series of multiple alleles in *Drosophila* (Fig. 8). If this working hypothesis proves tenable, it should be possible to achieve a better understanding of the reactions of growth-regulating enzymes in the plant.

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THE BALANCE BETWEEN SEXUAL AND APOMICTIC REPRODUCTION IN SOME HYBRIDS OF POTENTILLA[1]

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(Received December 21st, 1957)

I. INTRODUCTION

IN Scandinavia the collective species *Potentilla argentea* is represented by diploid, tetraploid, pentaploid and hexaploid cytotypes, which are all strictly apomictic (*cf.* A. and G. MÜNTZING, 1941, MÜNTZING, 1958). The hexaploids have been distinguished as a separate agamospecies by MARKLUND (1933), who points out that they correspond to *P. impolita* WB. According to the same author the Scandinavian diploids should belong to *Potentilla argentea* L. It is doubtful whether all Scandinavian diploids may be included in this species (MÜNTZING, 1958), and in more southern regions other diploids occur which deviate strongly. This is true of a strain obtained by me from the Botanical Garden of Basel, Switzerland and previously described under the denomination A—C (A. and G. MÜNTZING, 1941, p. 252—257). Taxonomically this strain seems to correspond to *P. argentea* L. var. *calabra* SER., but crossing experiments (A. and G. MÜNTZING, 1941, 1945) have demonstrated that it rather deserves the rank of a separate species. These crosses, using A—C as the female parent, resulted in true hybrids, which shows that this strain is sexual in contrast to the Scandinavian diploids of *P. argentea*. The sexuality is not complete, however, A—C also producing unreduced ovules, which are sometimes fertilized, but which in certain cross combinations give rise to maternal offspring. These different alternatives, of which the A—C strain is capable, are realized in different frequencies in different cross combinations and are dependent on the degree of genetical differentiation between the parents as well as on the degree of polyploidy of the male parent.

In crosses between A—C ($2n=14$) and hexaploid, strictly apomictic

[1] O. RENNER zum 75. Geburtstag gewidmet.

argentea strains hybrids could easily be produced, but of these hybrids only one single plant had the expected chromosome number 28. All the others were pentaploid ($2n=35$), resulting from fertilization of unreduced ovules of the A—C parent. Progenies of six different F_1 plants showed that reproduction in these F_1 plants was on an average intermediate between apomixis and sexuality. However, the different F_1 individuals, and even sister plants of the same cross combination, were very different in this respect and represented a rather continuous range from complete apomixis to complete sexuality. This diversity may be ascribed to the heterozygosity of both parents (A. and G. MÜNTZING, 1945).

Thus, in hybrids between A—C and hexaploid *argentea* the sexual and apomictic tendencies are rather evenly balanced, the specific genetic constitution of the different F_1 plants shifting the balance in one way or the other. Diploid hybrids between A—C and diploid *argentea* apomicts, on the other hand, were found to be completely or almost completely sexual, the only trace of apomixis being the formation of a single triploid F_2 plant in an otherwise exclusively diploid and strongly segregating F_2 generation. Thus, in crosses between A—C and apomictic *argentea* strains one single genome from the apomictic parent is not enough to overcome the sexuality of the other parent, but three genomes from hexaploid apomicts in combination with 1 or 2 genomes of the sexual strain are sometimes, but not always, sufficient to ensure a strictly apomictic mode of seed formation in the F_1 hybrids.

Under such circumstances it is of interest to study hybrids between A—C and tetraploid apomictic *argentea*, which will combine genomes for sexuality and apomixis in a new way in comparison to the other two crossing categories. The present paper deals with some hybrids of this kind and also with the mode of reproduction of some additional pentaploid hybrids from the crosses between A—C and hexaploid apomicts.

II. HYBRIDS BETWEEN A—C AND TETRAPLOID, APOMICTIC ARGENTEA

Hybrids of this cross combination were produced with some difficulty. Thus, in 1941 flowers of A—C were pollinated with the pollen of two different tetraploid apomicts (216 and 223), both from the province of Blekinge, South Sweden. The cross A—C×223 resulted in 78 seeds, which failed to germinate. The other cross, A—C×216, gave

16 seeds, 2 of which germinated and gave rise to purely maternal A—C plants. The crosses were repeated again in 1945, and this time 13 A—C flowers, pollinated with the pollen of the tetraploid apomict 216, gave 14 seeds. Two of these seeds germinated and gave rise to mature plants of similar appearance, which were found to be true F_1 hybrids.

Morphologically these hybrids (24—1 and 24—2) were more similar to the apomictic male parent than to their mother strain, A—C. They were cytologically examined and found to be triploid ($2n=21$), thus being the result of unions of reduced gametes from both parents. Both hybrids were quite male sterile. A pollen sample of 24—1 only contained empty and shrivelled pollen grains, and in 24—2 the anthers did not dehisce at all. Pollen fertility in 10 plants of the male parent biotype was found to range from 40 to 70 with an average of 56 per cent apparently good pollen grains. The A—C strain could not be examined at the same time but is known to have an average of more than 90 per cent good pollen (A. and G. MÜNTZING, 1941, p. 254, 1945, p. 50).

Though male sterile the triploid hybrids gave seeds after open pollination, these seeds resulting from spontaneous crosses with adjoining biotypes. In the first place the parent strains, surrounding the F_1 plants, may have delivered the pollen, but also hexaploid *argentea* strains may be involved. In 1948 a total of 240 seeds were harvested from F_1 plant 24—1 and 130 seeds from 24—2. The former seed batch gave rise to only 12 plants, the latter to 46 plants. In both cases, however, a pronounced degree of zygotic selection may have occurred. The F_1 plants being perennial, new seed samples were collected and germinated in 1952. Seventeen additional plants were now raised from 24—1, and 38 plants from 24—2. Altogether $29+82=113$ progeny plants were obtained.

The first thing to be observed in the offspring was a striking difference in appearance between the progenies of the two F_1 plants. The offspring of 24—1 (»Progeny A» in Tables 1—2) was on an average vigorous and rather uniform, the majority of the plants being similar inter se and also similar to the mother plant. The offspring of the other F_1 plant, 24—2, (»Progeny B» in Tables 1—2) differed strikingly by showing a strong variation and poor average vigour. A minority of the plants in Progeny B were supposed to represent the F_1 type. Chromosome counts were undertaken in a total of 113 plants, the following result being obtained (Table 1).

Of the 29 plants belonging to Progeny A, 17 were triploid like the mother plant, the majority of these plants being recognized as ma-

TABLE 1. *Somatic chromosome numbers in the offspring of triploid hybrids between sexual and apomictic Potentilla argentea.*

Category	21...	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42...	46...	50	51...	53...	62	n
Progeny A, 1949	5	—	—	5	—	—	—	—	—	—	1	1												12
» », 1952	12	1	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	2						17
Progeny A, total	17	1	—	5	—	—	—	—	—	—	3	1	—	—	—	—	—	2						29
Progeny B, 1949				1	2	1	1	—	3	10	15	5	—	—	1	1	1	1...	1	—	1...	1...	1	46
» », 1952				2	2	2	1	1	5	2	13	3	1	—	—	—	2	3	—	1				38
Progeny B, total				3	4	3	2	1	8	12	28	8	1	—	1	1	3	4...	1	1	1...	1...	1	84

ternal before the chromosome numbers had been determined. Of the remaining 12 plants 10 were euploid with either 28, 35 or 42 chromosomes and one plant was approximately pentaploid, having $2n=36$. One single plant, afterwards found to have $2n=26$, was clearly aberrant, having abnormal leaves and poor vigour. From these observations it may be concluded that most of the offspring of F_1 plant 24—1 had arisen by parthenogenetic development of unreduced ovules. In ten or eleven cases these ovules had been fertilized by pollen from diploid, tetraploid or hexaploid strains ($21+7=28$; $21+14=35$; $21+21=42$). The single plant with $2n=26$ must have originated from a reduced ovule with ± 12 chromosomes fertilized by a pollen grain from a tetraploid strain.

In the B progenies, representing the offspring of F_1 plant 24—2, not only vigour but also the chromosome numbers were extremely variable, ranging from 28 to 62 (Table 1). Not a single plant was triploid and, consequently, this F_1 plant entirely lacked the ability of producing offspring by parthenogenetic development of unreduced ovules. However, the high average level of the chromosome numbers strongly indicates that such ovules were present, but that they were fertilized. The maxima at 35 and 42 may be explained in this way, representing ovules with 21 chromosomes fertilized by pollen grains from tetraploid and hexaploid biotypes. The three plants having 28 chromosomes may also be the result of fertilization of unreduced ovules, the male parent in this case being diploid. This is uncertain, however, as there is a continuous range of aneuploids between 28 and 35. Most probably these aneuploids have arisen by fertilization of reduced ovules, generally having between 7 and 14 chromosomes, the male parent in this case being hexaploid. The plants with 36 and 37 chromosomes may have arisen from ovules having one or two chromosomes more than the somatic number or by the functioning of male gametes with more than 14 chromosomes. Meiosis in the p.m.c. of the tetraploid apomicts has not been studied but the markedly reduced pollen fertility (*cf.* above p. 147 and MÜNTZING, 1958) indicates meiotic irregularities. In a similar way the plants with chromosome numbers around 42 may have originated from unreduced ovules with one or a few chromosomes too little or in excess or from the occurrence of male gametes with chromosome numbers slightly deviating from 21. Meiotic irregularities have, indeed, been observed in a hexaploid *argentea* type (MÜNTZING, 1931).

The plants with chromosome numbers as high as 50 to 62 may have arisen from ovules having twice the somatic chromosome number, these

TABLE 2. *Plant weight in progenies of triploid hybrids between diploid sexual and tetraploid apomictic Potentilla argentea.*

Category	Plant weight in gr. 0	50	100	150	200	250	300	n	$\bar{X}$
Diploid parent	14	13	3					30	56.7
Tetraploid parent		2	8	16	5	1		32	167.2
Progeny A, total	4	—	2	4	3	3		16	159.4
Progeny A, plants with 2n=21	1	—	2	4	2	3		12	187.5
Progeny B, total	18	14	8	4	1			45	76.1
» », euploids	2	8	4	2	1			17	101.5
» », aneuploids	12	6	4	2				24	66.6

presumably being fertilized by pollen from diploid or tetraploid strains. It is also possible that some of these plants have originated by chromosome doubling at the first division of the fertilized egg cell.

At any rate, the contrast between the two progenies is quite clear, showing that the sister F_1 plants had quite different modes of reproduction. In plant 24—1 28 of the 29 plants came from unreduced ovules, most of which developed parthenogenetically. In plant 24—2, on the contrary, probably not a single plant arose by parthenogenesis, all ovules being fertilized. At least 30 of the 84 ovules involved were probably reduced and 50 plants may have arisen by fertilization of unreduced ovules. This, however, is a maximum value, some of the plants in this category probably having arisen from reduced ovules. Finally, 4 ovules had probably twice the somatic chromosome number and were fertilized by pollen grains from diploid or tetraploid strains.

In the material cultivated in 1949 *plant weight* was determined, the data obtained being given in Table 2. There is an obvious difference in vigour between the parent strains, the average value of the tetraploid parent being about three times as large as that of the diploid parent (167.2 and 56.7, respectively). The contrast between Progenies A and B is also striking, the average values being 159.4 and 76.1, respectively. Testing the significance of the difference with an analysis of variance, the quotient between the mean squares for between and within series was found to be 18.81, corresponding to a P smaller than 0.001.

In Progeny A, 12 of the 16 plants were maternal triploids. Their average vigour was found to be 187.5, a value higher than the average of the entire progeny (159.4). This illustrates the observed superiority of the maternal triploids over the plants with deviating chromosome

TABLE 3. *Chromosome numbers in F_2 progenies of A—C×hexaploid apomictic argentea.*

Progeny	2n of mother plant	Somatic chromosome number																	n
		14...	29	30	31	32	33	34	35...	49	50	51	52	53	54	55	56	57	
1945—660	35		2	1	4	—	1	1	1										10
—661	±34	1...	3	6	3														13
—662	±35		1	—	—	—	—	15	1										17
—663	±34							1	1...	1	1	2	2	2	3	1	5	1	20

numbers. In Progeny B the average vigour of the euploids with 28, 35 and 42 chromosomes was found to be larger than that of the aneuploids, the average values being 101.6 and 66.6, respectively. Testing the difference with an analysis of variance, a v^2 value of 4.51 was obtained, which corresponds to a P intermediate between 0.05 and 0.01.

This difference is of interest as it supports the view that the euploids are not only numerical euploids (some of which might have been formed by unbalanced and not necessarily complementary reduced gametes) but true euploids, arisen from fertilization of unreduced ovules.

III. HYBRIDS BETWEEN A—C AND HEXAPLOID, APOMICTIC ARGENTEA

In a previous paper (A. and G. MÜNTZING, 1945) six progenies of hybriods between A—C and hexaploid apomicts were described. These progenies represent three different cross combinations, A—C being hybridized with two different hexaploids from South Sweden (»A—B» and »arg. Dalby») and with one from Harz in Germany. The latter cross combination was represented by progenies of three different pentaploid F_1 plants, the mode of reproduction in these F_1 plants being strikingly different. Therefore, it seemed desirable to raise more F_2 material from other F_1 plants of the same cross combination. This was done from four new F_1 plants, the offspring after open pollination of these plants showing the chromosomal variation given in Table 3.

Evidently the four progenies are strikingly different with regard to chromosome variation, and hence each one must be considered separately.

1945—660. This progeny, represented by 13 individuals in the field, showed a strong segregation in morphology as well as vigour, some individuals being rather poor though not quite defective. Evidently, the

mother plant had been purely sexual, all the progeny plants being the result of fertilization of reduced gametes. Theoretically, it is possible that the plant with $2n=35$ may have arisen by parthenogenetic development of an unreduced ovule, though this is not very probable.

1945—661. This progeny, represented by 13 individuals, is in the main of the same type as the preceding one, 12 of the 13 plants evidently being the result of fertilization involving reduced gametes. These plants also showed a strong segregation in morphology and vigour. However, the plant with $2n=14$, appearing in this progeny, is of particular interest. According to the notes made before the chromosome numbers had been determined it was a »weak F_2 plant». Thus, it certainly belonged to the progeny and does not represent an admixture of some diploid *argentea* strain. As the mother plant had $2n=\pm 34$, the only possible explanation seems to be that this plant had arisen by parthenogenetic development of a reduced ovule, having $2n=14$. As the plant was weak, these 14 chromosomes evidently did not represent a true euploid condition but some kind of unbalance.

1945—662. In the field this progeny was represented by 31 individuals, which were all alike and represented a complete uniformity. The plant afterwards found to have 29 chromosomes had died at the seedling stage. Of the plants in the field 16 were examined later on with regard to their chromosome numbers, 15 having $2n=34$ and 1 $2n=35$. As the latter plant was not morphologically deviating, its correct chromosome number was probably 34. Hence, we may conclude that the mother plant was almost completely apomictic, only exceptional daughter plants being formed by fertilization of reduced ovules.

1945—663. This progeny consisted of 20 individuals, and in the field these plants showed only a moderate degree of morphological variation. A few clearly deviating individuals were observed, but most of the plants seemed to represent the maternal type. The strong chromosome variation actually occurring was therefore surprising. It shows, however, that the great majority of the offspring had been produced by fertilization of unreduced ovules, and only two plants were probably derived from the fertilization of reduced gametes. However, the chromosome numbers of the latter two plants (34 and ± 35) are such that parthenogenetic development of unreduced ovules may also have occurred.

The pentaploid hybrids between A—C and hexaploid *argentea* apomicts have rather good pollen (*cf.* A. and G. MÜNTZING, 1945, p. 54) in contrast to the male sterile triploid hybrids between A—C and tetra-

ploid apomicts. This pollen is not only morphologically good but has also been proved to be functional in two F_1 plants, from which F_2 progenies were raised after isolation of the F_1 plants. Therefore, in the present case it is likely that at least part of the plants having from 49 to 57 chromosomes are ± autotriploids in relation to the mother plants, having arisen by self-fertilization. The reduced male gametes will have variable chromosome numbers and this will explain the chromosomal variation obtained. This hypothesis is also in accordance with the weak morphological variation. The pentaploid chromosome complement of the unreduced ovules + the genetically similar chromosomes contributed by the pollen will lead to a predominantly maternal appearance, and at the relatively high chromosome number level (49 to 57) the addition or subtraction of a few chromosomes will not have much effect.

Though this seems to be a plausible explanation of the observed facts, it is not excluded that part of the offspring may have been produced by back crosses to hexaploid apomicts rather than by self-fertilization. This would explain the little peak at $2n=56$, which would correspond to 35 chromosomes from the mother and 21 chromosomes from a reduced pollen grain of an apomict with $2n=42$.

IV. SURVEY AND DISCUSSION OF THE MODE OF REPRODUCTION OF THE DIFFERENT HYBRIDS

A characteristic feature of the results obtained is the strikingly different behaviour of the individual hybrids, almost every F_1 plant giving a specific spectrum of variation in F_2, which reflects differences in the mode of reproduction. To facilitate supervision of the data obtained from the crosses between A—C and tetraploid as well as hexaploid apomicts Table 4 was elaborated. This table also includes data from 6 progenies of hybrids between A—C and three different hexaploid apomicts which were reported previously (A. and G. MÜNTZING, 1945).

From the table it may be seen that normal sexual reproduction involving the union of two reduced gametes (alternative *a* in the table) occurs in all but one of the twelve hybrids studied. One of the hybrids between A—C and hexaploid *argentea* has only apomictic seed formation. However, the degree of normal sexual reproduction in the other eleven hybrids is highly variable, ranging from 3.4 to almost 100 per cent. On an average 52 % of the offspring of the hybrids between A—C and hexaploid *argentea* had arisen in this way. Of the two hybrids between A—C and tetraploid *argentea* one had only a very low degree

TABLE 4. *Survey of the mode of reproduction in hybrids between sexual and apomictic strains of Potentilla argentea.*

Field number and cross combination	Chromosome number of F_1 plant	Fertilization		Parthenogenetic development		n	Percentages of			
		Female gametes		Female gametes						
		(a) reduced	(b) unreduced	(c) reduced	(d) unreduced		a	b	c	d
408 A—C × A—B	35	11	—	—	8	19	57.9	—	—	42.1
409 »	35	11	11	—	—	22	50.0	50.0	—	—
410 A—C × arg. Harz.	28	11	30	—	—	41	26.8	73.2	—	—
412 A—C × arg. Harz	34	—	—	—	20	20	—	—	—	100.0
413 »	35	88	—	—	1?	89	98.9	—	—	(1.1?)
1945—660 A—C × arg. Harz	35	9	—	—	1?	10	90.0	—	—	(10.0?)
» —661 »	±34	12	—	1	—	13	92.3	—	7.7	—
» —662 »	±35	1	—	—	16	17	5.9	—	—	94.1
» —663 »	±34	(2?)	18	—	—	20	10.0	90.0	—	—
414 A—C × arg. Dalby	±35	23	3	—	—	26	88.5	11.5	—	—
A—C × hexaploid apomicts, average percentages						277	52.0	22.5	0.8	24.7
Pentaploid hybrids, average						236	54.8	16.8	0.9	27.5
Progeny A, A—C × tetr.apom.	21	1	11	—	17	29	3.4	37.9	—	58.6
» B, »	21	84				84	100.0			
A—C × tetraploid apomicts, average percentages						113	70.7		—	29.3

of normal sexual reproduction (3.4 per cent). In the other hybrid this percentage was certainly much higher but in this case categories a and b could not be distinguished with certainty.

In the other plants estimations could be made of the b frequency, *i. e.* the frequency of fertilization of unreduced ovules. Such fertilizations occurred in six of the twelve progenies, the average percentage in the category A—C×hexaploid *argentea* being 22.5. In the single F_1 plant of A—C×tetraploid *argentea*, for which this value could be calculated, it was found to be as high as 37.9.

Parthenogenetic development of the ovules was found to occur with certainty in 5 progenies and with probability in 2 additional ones. The average percentages of such development was 28.4 in the category A—C×hexaploid *argentea* and 29.3 in A—C×tetraploid *argentea*. The latter value is the average of 58.6, occurring in one of the two F_1 plants representing this category and the total absence of parthenogenesis in the other F_1 plant.

Only in one case of 64 was there evidence of parthenogenetic development of a reduced ovule: otherwise parthenogenesis only occurred in unreduced ovules. It is possible, however, that this proportion has been changed by zygotic selection, haploids from reduced ovules certainly being less viable than individuals derived from the parthenogenetic development of unreduced ovules. Plant belonging to the first category will have low and generally aneuploid chromosome numbers, those of the second category being euploids with the same chromosome number as the vigorous mother strain. The single plant belonging to category c happened to have $2n=14$ but was not completely balanced.

Among the 12 progenies studied there are three categories present with regard to the ratio of the genomes from the sexual strain A—C and the apomictic male parents. Thus, in the triploid hybrids between A—C and tetraploid *argentea* the ratio between these genomes is 1 : 2. One of the hybrids between A—C and *argentea* Harz had $2n=28$ and evidently a ratio of 1 : 3 as to the genomes for sexuality and apomixis. Finally, in the pentaploid hybrids between A—C and hexaploid *argentea* the corresponding genome ratio is 2 : 3. If the difference between sexuality and apomixis is governed by several genes present in all the genomes, the highest degree of sexuality should be expected in the pentaploid hybrid with the 2 : 3 ratio and the highest degree of apomixis in the tetraploid hybrid with the 1 : 3 ratio. The triploid hybrids, representing the 1 : 2 ratio, ought to be intermediate.

This is really true of category a (fertilization of reduced gametes),

the average of the pentaploids being as high as 54.8 as compared to 26.8 in the tetraploid hybrid. The corresponding value of the triploid hybrids is not exactly known but is probably intermediate between 26.8 and 54.8. Otherwise it is not possible to find any correlation between genome ratios and mode of reproduction. It is true that in the tetraploid hybrid as much as 73 per cent of the ovules are unreduced, indicating a strong apomictic tendency, but these ovules all lacked the ability of parthenogenetic development in contrast to all or a large proportion of the unreduced ovules in some of the pentaploid hybrids. Specific genes rather than genomes must be responsible for these clear-cut individual differences. This does not mean that the genome ratios are without importance. It still remains a fact that diploid hybrids between A—C and diploid apomicts (genome ratio 1 : 1) are quite sexual (A. and G. MÜNTZING, 1945, pp. 49—52), whereas a higher proportion of genomes from apomictic parents on an average increases the apomictic tendencies. Superimposed on this, however, we have the effects of individual genes, which may influence the frequency of unreduced ovules and the ability of such ovules (or reduced ovules) to develop parthenogenetically.

HÅKANSSON (1946) has performed very thorough embryological investigations of various species and hybrids of *Potentilla*, including the tetraploid and pentaploid hybrids between A—C and hexaploid apomicts. With regard to progenies 408 to 413 (Table 4) he has already discussed the occurrence of a good accordance between the cytogenetic and embryological data. He also describes the embryology of the mother plants of progenies 1945—660 to 663, in which the chromosomal conditions have not been available until now. It is therefore of interest to note that also in these cases there is a quite good agreement between the embryological data, the chromosome counts and the morphological field observations. Thus, in Plant 2—3, mother of progeny 1945—660, HÅKANSSON found that macrospores were frequently formed but that now and then also unreduced embryosacs were developed. According to Tables 3 and 4 at least 9 of the 10 individuals studied had resulted from fertilization of reduced ovules, one plant with $2n=35$ possibly being maternal.

In Plant 2—8, mother of Progeny 1945—661, HÅKANSSON again found most of the embryosacs to be reduced and this is confirmed by the data in Tables 3—4. A point of special interest in this case is the plant with $2n=14$, which must have arisen by parthenogenesis in a reduced embryosac. This is quite exceptional but may have some connection with

the fact that in this mother plant HÅKANSSON noticed a higher number of mature embryosacs per ovule than in the other *Potentilla* plants studied. The presence of 4—5 embryosacs in the same ovule will probably facilitate the parthenogenetic development of some of these embryosacs, and perhaps especially if one of the embryosacs is fertilized and not the other ones in the same ovule. Otherwise, parthenogenetic development of reduced embryosacs is only known in a few cases in *Poa* and *Potentilla*, in which the absolute chromosome has been increased over the normal level (MÜNTZING, 1940, 1943, HÅKANSSON, 1943). In this connection it should be mentioned that in the pentaploid hybrid 1—5, mother plant of Progeny 408 (Table 4) HÅKANSSON (1946) made fixations 11 days after emasculation of mature flower buds. He could then observe that the eggcells in unreduced embryosacs had started to divide, whereas no tendency to division could be seen in the egg nuclei of reduced embryosacs. This is in accordance with the fact that this plant gave two kinds of offspring, maternal individuals arisen by the parthenogenetic development of unreduced ovules and other plants representing the result of fertilization of reduced ovules.

HÅKANSSON (*l. c.*) also made observations on the embryology of Plants 2—9 and 2—11, mother plants of the progenies 1945—662 and 663 (Tables 3—4). In the former plant his observations led to the conclusion that macrospores are never or seldom formed and, indeed, of the 17 daughter plants 16 had arisen by parthenogenesis in unreduced embryosacs and only one plant was the result of fertilization of a reduced embryosac. In the latter plant, 2—11, meiosis on the female side was observed to be generally inhibited, but in rare cases apparently reduced embryosacs with small nuclei were seen. In this case, however, the unreduced embryosacs did not develop parthenogenetically but were all (or almost all) fertilized (Tables 3—4, Progeny 1945—663).

The diploid strain A—C, used for the crosses, is predominantly sexual but also has the ability of forming unreduced ovules. HÅKANSSON (1946) studied this strain embryologically and verified that a low proportion of unreduced embryosacs are really formed. In the A—C strain these unreduced embryosacs function in certain wide crosses and may then either be fertilized or develop parthenogenetically. HÅKANSSON considers this to be an exception to the rule that unreduced ovules in the hybrids either have the ability of parthenogenetic development or fertilization. It is also indicated that unreduced embryosacs needing fertilization are developed from a more sexual part of the ovule than the more somatic unreduced embryosacs, which are capable of partheno-

genetic development. This may be true of the hybrids between A—C and hexaploid *argentea*, but in one of the triploid hybrids between A—C and tetraploid *argentea* we have an obvious exception to this rule. According to Tables 1 and 4 Progeny A, consisting of 29 plants, represents 17 cases of parthenogenetic development of unreduced embryosacs and 11 cases in which such embryosacs were fertilized. In addition, there is one plant arisen by fertilization of a reduced embryosac.

RUTISHAUSER and collaborators, working with Swiss material of *Potentilla argentea*, *verna* and other species, have made numerous crosses between different species or biotypes and have succeeded in producing a number of hybrids which have been carefully analysed with regard to embryology, mode of propagation, morphology and fertility. The earlier papers by RUTISHAUSER and other literature on the mode of reproduction of hybrids between more or less sexual and apomictic types have been reviewed by A. and G. MÜNTZING (1945) and by HÅKANSSON (1946). In more recent papers RUTISHAUSER (1947, 1948) has advanced the theory that somatic and generative apospory in the genus *Potentilla* are controlled by different genetic factors, having quantitative effects. Hybrids between such types will be sexual in contrast to occasional hybrids between parents having the same type of apospory. RUTISHAUSER also concludes that the genes for different ways of apomictic seed formation are recessive in single dose but may become more and more epistatic if the number of genomes is increased. This view is in harmony with the fact that species hybrids between sexual and pseudogamous types of *Potentilla* are aposporous only when the apomictic parent has contributed a larger number of genomes than the sexual one (CHRISTOFF and PAPASOVA, 1943).

The data discussed in the present paper are only partly in accordance with this theory, as is evident from a study of Tables 1, 3 and 4. It is also doubtful whether the distinction between somatic and generative apospory can really be upheld in *Potentilla*. RUTISHAUSER (1948) finds *e. g.* that in *P. verna* no. 15 somatic as well as generative apospory may occur in the same plant, and RUTISHAUSER and HUNZIKER (1954) state that the difference between the two types of apospory is quantitative rather than qualitative. Thus, though some progress has been made, many problems concerning the genetic basis of apomictic seed formation are still unsolved.

SUMMARY

(1) Hybrids between a diploid sexual strain (A—C) of *Potentilla argentea* and tetraploid apomicts of the same collective species were produced. The hybrids were triploid and markedly sterile, but offspring after open pollination could be obtained.

(2) The two triploid sister plants studied were found to have different modes of reproduction. In one plant all the embryosacs, reduced as well as unreduced ones, were fertilized, in the other plant 59 per cent of the daughter plants came from the parthenogenetic development of unreduced embryosacs. However, 38 per cent of the plants were derived from unreduced but fertilized embryosacs and one individual was the result of fertilization of a reduced egg cell.

(3) Four new F_2 progenies of pentaploid hybrids between A—C and a hexaploid apomictic strain of *argentea* have also been studied. Two of the F_1 hybrids were found to have almost normal sexuality, most of the offspring resulting from fertilization of reduced egg cells. In one of these progenies, however, a plant appeared, which must have arisen by the parthenogenetic development of a reduced egg cell. In another progeny almost all the daughter plants were derived from the parthenogenetic development of unreduced embryosacs, whereas in the fourth progeny at least 90 per cent of the plants were derived from fertilization af unreduced embryosacs.

(4) Taking also previous results into consideration, it is concluded that the mode of reproduction of hybrids between sexual and apomictic *P. argentea* is not only determined by the ratio of genomes from the sexual and apomictic parents but also, and sometimes very conspicuously, by the action of specific genes.

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THE RECOVERY PHENOMENON AFTER IRRADIATION IN DROSOPHILA MELANOGASTER

IV. SPONTANEOUS RECOVERY OF IRRADIATED CHROMOSOMES VERSUS DIFFERENTIAL SENSITIVITY

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(Received March 28th, 1957)

INTRODUCTION

IN the first paper in this series LÜNING and HANNERZ (1957) examined the mechanism of the action of the oxygen concentration on X-ray induced chromosome breaks in spermatozoa of *Drosophila melanogaster*. They concluded in agreement with BAKER and VON HALLE's (1953, 1955) suggestion that the lower rate of chromosome aberrations induced at anoxia compared to that in air was due to a preferential rejoining of broken ends in the original order—recovery. A similar mechanism seemed also to be at work in reducing the rate of chromosome aberrations in sperm kept by the males for 24—48 hours after irradiation in air compared to those kept less than 24 hours. The latter suggestion was based on the results of elimination of ring-X chromosomes. It presupposes that primarily the difference is similar between the two spermbatches from ring-X males as has been found in males with rod X-chromosomes. In order to establish the existence of such a recovery process, which will be called spontaneous recovery to distinguish it from the oxygen dependent recovery, it has to be shown that there exist differences in the rates of chromosome breaks available for rearrangements in the two spermbatches from ring-X males. Translocations between chromosomes II and III could be used as indicators of such chromosome breaks. A study of that type will be reported below.

With the establishment of a spontaneous recovery process, arises the question of its relation to the findings of LÜNING (1952), that there is a

difference in sensitivity to X-rays in various stages of spermiogenesis. That problem will also be dealt with.

MATERIAL AND METHODS

As was stated above the establishment of a spontaneous recovery process requires an analysis of the rates of chromosome breaks available for rearrangements in the 1st versus the 2nd spermbatch from males of the same type as was used by LÜNING and HANNERZ when putting forth the hypothesis, viz. $X^{c^2};sc^8Y$ males from a $y\ f:=;sc^8Y \times X^{c^2};sc^8Y$ stock. Information of this could be derived from studies of II—III translocations. The occurrence of these was studied by the conventional genetic technique, using *cn bw*;e^{11} females. 3—4 days old $X^{c^2};sc^8Y$ males were irradiated (3240 r) and were subsequently mated to *cn bw*;e^{11} females in a population cage for 24 hours and were then mated in the same way to a new group of females for another 24 hours. The females were allowed to continue egglaying in vials for two 3-day periods. The F_1 males were mated singly to 3 *cn bw*;e^{11} females. The F_2 offspring was analyzed without etherization and all non-translocations were recorded. Suspected translocations were etherized and examined. The occurrence of only two classes, viz. *cn bw*;e^{11} and wild type was used as criterion of translocations. In a few vials with a total number of flies less than 13 the wild type males were mated to new virgin *cn bw*;e^{11} females and counts were made in the F_3 generation.

In order to study the possible co-existence of a spontaneous recovery process and differential sensitivity between stages in spermiogenesis of *Drosophila* the same technique could be used as was described by LÜNING and HANNERZ. They discussed in detail the background of the use of ring-chromosomes as indicators of recovery versus differential sensitivity. Here it suffices to say that at recovery there are much smaller changes in the rates of eliminations in the two lots considered when using ring-chromosomes than rod-chromosomes, while with differential sensitivity there will not be any differences between the results in ring- and rod-chromosomes. In their experiments $X^{c^2};sc^8Y$ males were irradiated and mated to *y w sn* females. Among the normally grey offspring appeared yellow (*y w sn*) males which were supposed to be X0. The rate of these yellow males were taken as a measure of the rates of eliminations of X- and Y-chromosomes. The confirmation of their results by means of sexlinked recessive lethals (LÜNING and

SÖDERSTRÖM, in press) justify the continued use of these yellow males as indicators of chromosome-eliminations.

In the present experiments $X^{c2};sc^{8}Y$ males (of the same origin as in the translocation experiment) were collected within 24 hours after eclosion. They were in some experiments irradiated immediately, while in others they were stored for 3 days in vials with about 100 males in each. The males were given a dose of 1080 r (2 min.) and were immediately mated in population cages to *y w sn* females. (These females were taken from a stock maintained as $y\ w\ sn \times y\ w\ sn;sc^{8}Y$.) After 24 hours all the flies were taken out of the cage and the females were allowed to oviposite for two 3 day periods. The procedure was in some experiments repeated twice again with 48 hour mating periods. The females from the mating period corresponding to the 4th and the 5th day after irradiation of the males, were discarded, while those from the 6th—7th day mating were bred. The F_1 offspring was inspected and the number of grey females and males as well as yellow males were recorded.

RESULTS

A. Translocations

The purpose of this experiment was to study the rates of breaks available for rearrangements in the 1st versus the 2nd spermbatch from $X^{c2};sc^{8}Y$ males. As LÜNING and HANNERZ had irradiated their males when 3—4 days old, the same age was chosen also in this test and all precautions were taken to keep the conditions as identical as possible with those at the previous test of eliminations.

The results are presented in Table 1. It can immediately be seen that the yield of translocations after the 2nd day mating was much less than

TABLE 1. *Tests of II—III translocations induced in sperm from* $X^{c2};sc^{8}Y$ *males.*

The males were given 3240 r when 3—4 days old. They were immediately mated to *cn bw*;e^{11} females for 24 hours (ser. 1) and were then transferred to new females for another 24 hours, (ser. 2). The F_1 males were mated singly to *cn bw*;e^{11} females. The F_2 offspring was analyzed.

Series	Tested spermatozoa	II-III translocations	
		Abs. no.	%
1	571	61	10.7
2	981	64	6.5

that of the 1st day. The difference is significant, P=0.01. This is, however, of less interest than the ratio. Adjusting for a 3/2 power relation between the rate of chromosome breaks and the rate of translocations it is possible to estimate the relation between the rates of breaks in the two spermbatches. The rate of breaks available for rearrangements in the 2nd spermbatch is about 70 % of that in the 1st. This figure agrees very well with those previously obtained in other stocks with rod chromosomes. Hence there are no indications that the X^{c^2};sc^8Y males should differ from other stocks tested as regards to the difference between the first two spermbatches. This conclusion is of importance for the interpretation of the results of elimination of X- and Y-chromosomes in sperm from X^{c^2};sc^8Y males, which were presented by LÜNING and HANNERZ. In that paper it was stated that the results seemed to be in agreement with the recovery hypothesis, viz. that in the 2nd spermbatch fewer breaks were available for rearrangements due to the fact that a proportion of them were bound to rejoin in the original order—recovery. That statement presupposes that the X^{c^2};sc^8Y stock does not differ from other stocks as regards to the primary differences between the 1st versus the 2nd spermbatch. As this supposition has now been confirmed the statement of the existence of a spontaneous recovery process is strengthened.

B. Recovery and differential sensitivity in spermiogenesis

The establishment of the existence of a spontaneous recovery process as well as of a recovery process dependent upon the oxygen concentration during the treatment raises the question whether there also exists differential sensitivity between various stages in spermiogenesis as was proposed by LÜNING (1952). As a support of the hypothesis of a differential sensitivity, there was presented a study of eliminations of ring-X chromosomes. That experiment was, however, a very small one. For that reason it seemed desirable to repeat that experiment using the stocks by which LÜNING and HANNERZ had gotten results in favour of the existence of a recovery process in sperm from the 2nd day after irradiation. As the strongest differences between various stages in spermiogenesis were obtained after irradiation of newly hatched males (0—1 day old, LÜNING, 1952) the X^{c^2};sc^8Y males were collected when less than 24 hours old and irradiated (1080 r). They were immediately mated to *y w sn* females and then to new females after 24, 72 and 120 hours and were discarded 168 hours after irradiation. Hence the

TABLE 2. *Offspring from crosses y w sn×X^{c2};sc^{8}Y. Parts of the males were irradiated (1080 r) when 0—24 hours old; the rest were used as control.*

The irradiation series is called I with a subscript indicating the days of mating after irradiation. The control series is called C and also with subscript analogous to those in the irradiation series.

Series	Total F_1 flies	Yellow males (XO)	
		Abs. no.	%
I 1	13395	126	0.94
I 2—3	13612	150	1.10
I 6—7	9004	335	3.72
C 1	10564	38	0.36
C 2—3	12851	49	0.38
C 6—7	6160	26	0.42

mating periods are the 1st, 2nd—3rd, 4th—5th, and the 6th—7th day after irradiation. The last mating period corresponds to the period at which the maximum rate of chromosome aberrations is expected. Besides the irradiation series there was also a parallel series with untreated males which were mated at the same intervals as their irradiated brothers. These precautions were taken in order to eliminate possible differences not associated with the effect of the X-ray treatment.

The results are presented in Table 2. The control series show very good constancy throughout the experiment. If we then compare Ser. I 1 and Ser. I 2—3 with Ser. I 6—7 it is evident that there is a strong difference. To get an idea about this difference a comparison can be made with the results of LÜNING and HANNERZ. They found after irradiation of 3—4 days old males with 3240 r that in the 1st sperm-batch there were induced 2.93 % yellow males, this can be compared to the rate 3.78 in Ser. I 6—7, which was only given 1080 r. To induce the same rate of yellow males in sperm to be deposited the 1st day as in sperm deposited the 6th—7th day, a dose more than three times higher than in the latter period is needed. That conclusion agrees very well with the findings of LÜNING (1952), who found a factor slightly below 4 when using rod-X chromosomes. It was said above that agreement between the results from ring- and rod-X chromosomes should indicate differential sensitivity rather than recovery. Hence it must be concluded that the difference in the rate of chromosome aberrations between the two stages studied is due to differential sensitivity. Consequently both

TABLE 3. *Offspring from crosses y w sn* ♀×X^{c^2};sc^8Y.

The males were stored without females for 3 days. Parts of the males were irradiated (1080 r) and the rest were used as control. The designation of the series is identical with that in Table 2.

Series	Total F_1 flies	Yellow males (XO)	
		Abs. no.	%
I 1	16466	240	1.46
I 2—3	15751	159	1.01
I 6—7	11699	262	2.24
C 1	15763	77	0.49
C 2—3	14999	83	0.53
C 6—7	6018	24	0.40

recovery processes and differential sensitivity in spermiogenesis exist together.

LÜNING (1952) also showed that the difference between the rates of aberrations induced in various stages in spermiogenesis was much less than when the males were stored some days prior to the treatment. Also in order to elucidate this, it seemed worth while to repeat the experiment presented in Table 2 but using 3—4 days old males instead of newly hatched males. The procedure was the same as in the previous experiment. The results are presented in Table 3. Also in this case there is a higher rate of eliminations in Ser. I 6—7 than in Ser. I 1 and Ser. I 2—3 but when compared to the results in Table 2 it is evident that the difference is much less than after irradiation of 0—24 hours old males. This is in very good agreement with the findings of LÜNING (1952) by rod-X chromosomes.

It has been proposed by MOSSIGE (1955) and BATEMAN (1956) that there is a storing of sperm in males kept without females some days prior to the irradiation and hence that there can be a mixing of sperm from different stages. That would possibly explain the lower rate in Ser. I 6—7 from males irradiated when 3—4 days old (Table 3) versus that in Ser. I 6—7 from 0—1 day old males (Table 2). LÜNING (1952) on the other hand suggested that there was no considerable storing of sperm in young males. From that point of view the difference between the two Ser. I 6—7 referred to were due to variations in sensitivity depending on the age of the males at treatment.

In order to get some information on that problem, another experiment was made of the same type as those presented in Tables 2 and 3. This

TABLE 4. *Offspring from crosses y w sn*$\times$X^{c2};*sc*^{8}Y.

The males were stored *with* females for 3 days. Parts of the males were irradiated (1080 r) and the rest were used as control. The designation of the series is identical with that in Table 2.

Series	Total F_1 flies	Yellow males (XO)	
		Abs. no.	%
I 1	13039	226	1.73
I 2—3	22876	297	1.30
I 6—7	10022	330	1,77
C 1	9254	39	0.42

time the males were also irradiated at an age of 3—4 days but they had from eclosion been kept *with* an excess of females in a population cage. Thus a storing of sperm in the males was prohibited. After irradiation with 1080 r they were subsequently mated. The mating scheme was the same as in the previous experiments. There was also a control series which, by accident, consists of the 1st day mating only. The results are presented in Table 4. The crucial case is Ser. I 6—7 which will be compared with the corresponding series in Tables 2 and 3. If the comparatively low rate in Ser. I 6—7 in Table 3 was due to a mixing of sperm of more or less sensitive stages caused by the storing of the males *without* females for 3 days prior to the irradiation a higher rate in Ser. I 6—7 in Table 4 would be expected, as in that case the males had had females all the time from eclosion. As is seen this is not the case rather the reverse, (2.24 vs. 1.77). Thus the results in Table 4 do not give any support to the hypothesis of mixing of sperm as reason for the lower rate of chromosome eliminations in Ser. I 6—7 from males irradiated when 3—4 days old as compared to that from 0—1 day old males. For that reason it is more probable that there is a real difference in sensitivity dependent on the age of the males at irradiation as concerns stages supposed to be spermatids. It ought to be emphasized that there is nothing in favour of a supposition of a recovery process causing this difference.

Acknowledgements. — The author is deeply indepted to Professor GERT BONNIER for many helpful discussions, to Miss B. SCHUWERT for technical assistance and to Mr. and Mrs. RAMEL for revising the English text. — The present study was supported by the Swedish Natural Science Research Council.

SUMMARY

(1) The hypothesis of a spontaneous recovery process in sperm irradiated in air was based on studies of eliminations of X- and Y-chromosomes in sperm deposited the 1st versus the 2nd day from two types of males, viz. X^{c^2};sc^8Y and Canton S;sc^8Y (see LÜNING and HANNERZ, 1957). This conclusion presupposes that the ratio of chromosome breaks available for rearrangements in the two spermbatches under consideration was of the same order in both types of males. In order to test this X^{c^2};sc^8Y males were irradiated and the rates of II—III translocations in the 1st versus the 2nd spermbatch was examined by the conventional genetic technique. It is shown that the ratio was of the same order as in other stocks tested. Hence the hypothesis of a spontaneous recovery is further strengthened.

(2) The hypothesis of differential sensitivity in various stages of spermiogenesis as proposed by LÜNING (1952) has been reinvestigated. The same technique was used as in the establishment of the spontaneous recovery process, viz. eliminations of X- and Y-chromosomes in sperm from X^{c^2};sc^8Y males. It is concluded that the high rate of chromosome aberrations induced in stages supposed to be spermatids was due to a higher sensitivity. Furthermore it is shown that the sensitivity in these stages depended on the age of the males at treatment. Hence, the coexistence of a spontaneous recovery and of differential sensitivity in spermiogenesis in *Drosophila* has been demonstrated.

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THE RECOVERY PHENOMENON AFTER IRRADIATION IN DROSOPHILA MELANOGASTER

V. THE RELATION OF VIABILITY MUTATIONS TO BREAKAGE OF CHROMOSOMES

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IN a study by LÜNING and SÖDERSTRÖM (1957) the relation between recessive lethals and chromosome breaks in *Drosophila melanogaster* was discussed. They concluded that breakage *per se* and rejoining by recovery were not the origin of recessive lethals. This conclusion was based on the assumption of the existence of a recovery process after irradiation at anoxia as proposed by BAKER and VON HALLE (1953, 1955) and confirmed by LÜNING and HANNERZ (1957). The latter inferred that sperm giving viable zygotes would contain more primary breakage points if there had been a recovery process than in sperm where there had not been any or less recovery. In spite of the higher rate of primary breakage points in viable zygotes from sperm irradiated at anoxia, fewer recessive lethals are induced than in sperm irradiated in air atmosphere. Furthermore LÜNING and SÖDERSTRÖM showed that when the whole dose was given at anoxia a proportion of the primary changes (potential lethals) did not manifest as lethals but instead recovered. However, it could not be concluded that they reverted to the original unmutated state.

The recovery of potential recessive lethals as well as the occurrence of a higher rate of primary breakage points in sperm in which there had been a recovery process, raises the question whether those changes when not ending up as recessive lethals will show up as mutations with slight effects on viability—detrimentals. In order to study this a small experiment was undertaken and will be presented below.

MATERIAL AND METHODS

In this experiment males were taken from a *w* stock which was made isogenic for the X-chromosome. They were mated to Muller-5 (sc^{S1} *B InS* w^a sc^8=M5) females from a stock isogenic for the M5 chromosome.

The *w* males were collected when less than 24 hours old and were stored for 3 days without females. When 3—4 days old they were irradiated with 3240 r X-ray and were immediately mated for 24 hours, and in one series also for another 24 hours. The F_1 daughters were collected when virgin and were then mated singly to 3 M5 males from the isogenic M5 stock. From each of them a strain was made up with a record number of its own. After four days the flies were transferred to new vials. From each number, 10 heterozygous *w*/M5 daughters were collected and were mated singly to their brothers for 4 days. The F_2 offspring was analyzed and the number of *w* and M5 males was recorded. The minimum for each tested chromosome was 300 males.

RESULTS

The purpose of this investigation was to study the rate of viability mutations in sperm which could contain recovered chromosome breaks and recovered potential lethals and compare this with the corresponding rate in sperm with less or no recovery. In the introduction, the fact that chromosome breaks could recover after irradiation at anoxia was referred to, LÜNING (in press), has also presented data indicating that there is also a spontaneous recovery process in sperm irradiated in air and kept by the males for at least 24 hours. The sperm deposited the first 24 hours after irradiation of the latter males was supposed to have no (or less) recovery. Hence three groups of sperm could be studied: one without (or with less) and two with recovery. To get the first group the *w* males irradiated in air were mated to M5 females for 24 hours, Ser. A 1. These males were then mated to new females 24—48 hours after irradiation, Ser. A 2.

In the last series the males were irradiated at anoxia and were mated for the first 24 hours only, Ser. B.

In Fig. 1 the distribution of the percents of *w* males $\left(\frac{w \cdot 100}{w + M5}\right)$ are given for the three series from 0 to 40 % each interval corresponds to 5 % but from 40 % each interval is 1 % only.

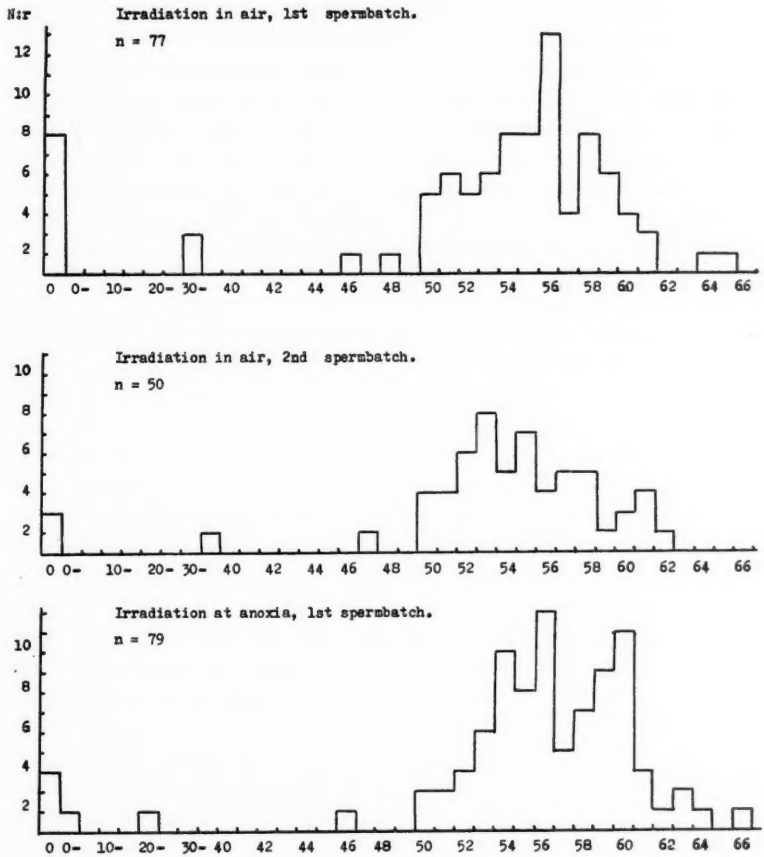

Fig. 1. Diagram showing the number of tests against different percentages of *w* males $\frac{w \cdot 100}{w + M5}$ among the offspring from *w*/M5 females. *w* males were irradiated (3240 r) in air or at anoxia and were mated to Muller-5 (=M5) females for 24 hours. In one series the males were also mated for another 24 hours. Interval »0» represents recessive lethals. Intervals »0—» to »30—» represent 5 % each. From 40 each interval is 1 %.

As the number of chromosomes tested in each series is very low, it is not possible to draw any conclusion about eventual differences in the rates of viability mutations which strongly decrease the viability. The recent studies of LÜNING and JONSSON (in press) have confirmed the results of TIMOFÉEFF-RESSOVSKY (1935) and KERKIS (1938) as regards to the occurrence of high rates of mutations which only have a slight effect on the viability and which cannot be individually identified. Their existence can only be proved by statistical means, as changes of the distribution within the quasinormal region. The majority of mutations affecting viability fell within this region. Thus it is of special interest to analyze the distribution of the three series in Fig. 1. The means in the quasinormal region (above 45 % *w*) males are: Ser. A 1=55.29; Ser. A 2=54.83 and Ser. B=56.64. A comparison between Ser. A 1 and Ser. A 2 showed no statistical difference, while a comparison between Ser. A 1 and Ser. B showed a significant difference, $0.05 > P > 0.01$. As is indicated by the mean values the difference means that there is a better viability in the cultures after irradiation at anoxia than in those after treatment in air. In the introduction the conclusion by LÜNING and HANNERZ, and LÜNING and SÖDERSTRÖM was referred to that the chromosomes in sperm irradiated at anoxia (Ser. B) will contain more primary breakage points than those irradiated in air, (Ser. A 1). As the studies of viability mutations have indicated that there seem to be fewer mutations influencing viability in Ser. B than in Ser. A 1 and the difference between Ser. A 1 and Ser. A 2 is insignificant the conclusion can be drawn that breaking of the chromosomes *per se* and rejoining of broken ends by recovery do not even bring about the origin of mutations with slight effects on viability. Hence it has not been possible to demonstrate any genetic effects of breaking *per se* and rejoining by recovery.

SUMMARY

Along with the demonstration of recovery (rejoining of broken chromosomes in original order) in *Drosophila melanogaster* sperm, there arose the question whether breakage *per se* or rejoining by recovery had genetical consequences.

Recovery of chromosome breaks was supposed to occur after irradiation at anoxia and in sperm irradiated in air and kept by the males for 24—48 hours. Sperm inseminated within the first 24 hours after irradiation in air showed no (or at least less) recovery. In order to study the

effect on viability mutations males from a *w* stock (isogenic for the X-chromosome) were irradiated in air or at anoxia and were mated to Muller-5 (M5) females (also isogenic for this X-chromosome). The F_1 daughters were mated singly in the conventional way; 10 *w*/M5 F_2 females from each culture were mated singly and the F_2 offspring was analyzed as for the number of *w* and M5 males. The material was too small to draw any conclusions about mutations with strong effects in the viability. As regards to the quasinormal region, there was no significant difference between the two spermbatches irradiated in air while the viability in offspring after irradiation at anoxia was significantly higher. From these results conclusion has been made that breakage *per se* or reunion by recovery seems not to have any genetic effect.

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SELF-INCOMPATIBILITY IN RYE

III. HOMOZYGOSITY FOR INCOMPATIBILITY FACTORS IN RELATION TO VIABILITY AND VEGETATIVE DEVELOPMENT

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I. INTRODUCTION

IN a previous paper (LUNDQVIST, 1956), self-incompatibility studies of diploid rye in 1954 and 1955 were reported, and data were presented from three population clones of the commercial variety Steel-rye, displaying different degrees of incompatibility strength. Since that time, employing the same experimental techniques, a number of additional families belonging to two of these clones have been investigated. All of these families are derived from compatible combinations, and most of the latter are intrafamily sib combinations. As before, an exact assignment of genotypes for the whole material has not been attempted; for the present purposes it has been sufficient to consider in relation to expectation the number of incompatibility groups, obtained in the various cases. Previous data warranted the conclusions (1) that self-incompatibility in rye is gametophytically controlled by two independently segregating, probably multiallelic, loci, (2) that both factors of the pollen have to be matched in the style for the pollen to react incompatibly, and (3) that a single incompatibility factor common to pollen and style will cause no functional disadvantage to the pollen carrying it.

It is specifically characteristic of this type of incompatibility system that individuals, homozygous at one or the other of the incompatibility loci, may occur naturally in a population. By itself, heterozygosity not being obligatory for these loci, there might be no reason to expect here an especial accumulation of deleterious recessive genes in adjacent chromosome regions, such as may occur where production of homozygosity is prevented. From these considerations, data on the effect of

homozygosity for incompatibility factors on viability and vegetative development might be of interest in the discussion of the origin and evolution of an incompatibility system with the outlined way of action.

In the following, plants of the double heterozygote genotype (het het) will be contrasted with plants belonging to the single heterozygote category (ho het), where the four possible genotypes have been lumped together, without any attempt to distinguish between them. The ho ho category was too rarely represented to deserve special consideration, and will therefore be omitted. Only the 28 families, where both het het and ho het but no ho ho plants were theoretically expected, will be considered, but they constitute the bulk of the families. For the families studied in 1954—1955, *viz.*, Nos. 1—2, 4—9, 11—12, and 15—20, data have been already given (LUNDQVIST, 1956, Table 6 and Table 7). As this presentation is not quite adequate for the present purpose, these families have been resummarized in Table 1 together with the additional families Nos. 26—41, investigated in 1956—1957.

II. REVIEW OF FAMILIES 26—41. NEW DATA ON THE FUNCTIONING ABILITY OF POLLEN WITH ONE FACTOR MATCHED IN THE STYLE

Offspring from cross combinations such as $ho_a ho \times ho_b het$ (*e. g.*, $S_{1.1}Z_{3.3} \times S_{2.2}Z_{3.4}$) and ho ho×het het (*e.g.*, $S_{1.1}Z_{3.3} \times S_{1.2}Z_{3.4}$) permit a crucial genetical test of the fertilizing ability of pollen with one incompatibility factor common to the style, when competing with comparable pollen with no factor matched. Data obtained from clone 21 in families 12 and 15, respectively, gave a strong indication that the latter category of pollen had no functional advantage, the segregation ratios showing no significant disturbance (LUNDQVIST, 1956). The numbers of plants analysed were small, however, and the remaining materials were therefore grown and tested as families 26 and 28, the data being entered in Table 1 *a* and 1 *b*, respectively. In addition, two new families, Nos. 38 and 39, derived from the cross combination ho ho×het het within clone 21 are given in Table 1 *b*.

Families 12 and 26 lumped together, segregate into 18 het het and 16 ho het plants ($\chi^2=0.118$; P=0.8—0.7). Families 11 and 27 lumped together from the reciprocal cross combination give 10 het het and 10 ho het plants. When ho ho is pollinated by het het, families 15 and 28, pooled segregate into 8 het het, 5 ho het, and 4 het ho plants, the

TABLE 1. *Material of diploid Steel-rye studied in 1954—1957. Origin and constitution of families segregating het het and ho het but no ho ho plants.*

	Offspring			Parental plants		Nos. offspring				Ratio het het/ho het	
	Family No.	Year	Clone No.	Nos.	Genotypes and mating category	groups		plants		Expected	P of observed deviation
						obs.	exp.	het het	ho het		
a.	12	1955	21	10: 16×2	C. ho_a ho×ho_b het	2	2	3	9	1 : 1	0.15
	26	1956	21	»	C. »	2	2	15	7	»	0.13
	11	1955	21	10: 2×16	C. ho_b het×ho_a ho	2	2	4	6	»	0.75
	27	1956	21	»	C. »	2	2	6	4	»	0.75
b.	15	1955	21	10: 8×14	D. ho ho ×het het	3	3	2	2+2	1 : 2	1.00
	28	1956	21	»	D. »	3	3	6	2+3	»	0.20
	38	1957	21	37[1]: 11×32: 14	D. »	3	3	6	6[2]	»	0.36
	39	1957	21	36[1]: 16×29: 9	D. »	3	3	6	9+9	»	0.52
c.	4	1954	16	S_1 16: 6×5	G. ho het×het ho	2	2	7	3	1 : 1	0.34
	5	1954	16	S_1 16: 5×6	G. »	2	2	7	6	»	1.00
	6	1955	16	3: 17×9	G. »	2	2	4	2	»	0.69
	29	1956	21	15: 4×5	G. »	2	2	5	4	»	1.00
	30	1956	21	15: 4×6	G. »	2	2	3	5	»	0.73
	41	1957	21	28: 9×7	G. »	2	2	8	7	»	1.00
d.	9	1955	16	7: 7×4	F. ho_a het×ho_b het	3	3	2	7[2]	1 : 1	0.18
	19	1955	21	17: 13×1	F. »	3	3	2	1+4	»	0.45
	20	1955	21	18: 8×1	F. »	3	3	6	3+3	»	1.00
	32	1956	21	16: 2×1	F. »	3	3	11	8[2]	»	0.65
	33	1956	21	16: 1×2	F. »	3	3	10	7[2]	»	0.63
	34[3]	1956	21	16: $\begin{Bmatrix}1\times2\\2\times1\end{Bmatrix}$	F. »	3	3	5	2+2	»	1.00

e.	1	1954	3	S_1 3: 3×P3	E. ho het × het het	3	3	9	1+3	1 : 1	0.27
	7	1954	16	S_1 16: 5×P16	E. »	3	3	6	2+1	»	0.51
	16	1955	21	10: 12×14	E. »	3	3	4	4+3	»	0.55
	17	1954	21	S_1 21: 9×P21	E. »	3	3	4	4+3	»	0.55
	18	1954	21	S_1 21: 11×P21	E. »	3	3	6	5+3	»	0.79
	35[3]	1956	3	1: {3×4 / 10×11}	E. »	3	3	11	5+2	»	0.48
f.	2	1955	3	1: 8×1	F. ho_b het or ho_a het × or E. het het	3	3	3	3+3	1 : 1	0.51
g.	8	1954	16	S_1 16: 6×P16	Unknown × het het	2	3	8	6	Unknown	

C.—G. refer to the mating categories of Scheme 1 in LUNDQVIST, 1956.

[1] Families 36 and 37 (not published here) were obtained from selfing two ho het plants obtained within clone 21. Plants 37: 11 and 36: 16 both proved to be homozygous at both loci and were crossed with het het plants derived from the same clone No. but belonging to other families.

[2] A complete grouping was not possible, but not less and not more than 3 groups could be distinguished.

[3] Family constituted by plants unaffected by a colchicine treatment in the autumn of 1955. The plants were kept in boxes in the greenhouse for the whole time.

ideal, undisturbed, segregation giving 5.67 plants in each group (the deviation corresponding to $\chi^2 = 1.523$ and $P = 0.5—0.3$). Families 38 and 39 taken separately display no significant disturbance of the segregation, and pooled they give 12 het het and 24 ho het, (lumped together), plants, which exactly corresponds to the ideal ratio.

The results of the continued analysis of families 15 and 28 are entered in Table 1 *c*. The assumption that they were derived from the cross combination ho ho × het het has the corollary that the two reciprocally compatible ho het groups distinguished here are heterozygous at different loci (*e. g.*, $S_{1.1}Z_{3.4}$ and $S_{1.2}Z_{3.3}$, respectively). In the progeny from these groups crossed, only two genotypes will be obtained ($S_{1.1}Z_{3.4} \times S_{1.2}Z_{3.3} \rightarrow S_{1.2}Z_{3.3} + S_{1.2}Z_{3.4}$). As expected, families 29, 30 and 41 segregate into only two groups. It may be concluded that families 15 and 28 clearly demonstrate that, at least in the case of these incompatibility alleles, pollen with one factor common to the style had no functional disadvantage, irrespective of the locus.

It may be of interest here to see that from the cross combination ho het × het het the offspring segregate into three groups, of which the two reciprocally compatible ho het groups, combined, will in turn give rise to three groups in their offspring (*e. g.*, $S_{1.1}Z_{3.4} \times S_{1.2}Z_{3.4} \rightarrow S_{1.2}Z_{3.3} + S_{1.2}Z_{4.4} + 2\,S_{1.2}Z_{3.4}$. Then $S_{1.2}Z_{3.3} \times S_{1.2}Z_{4.4} \rightarrow S_{1.1}Z_{3.4} + S_{2.2}Z_{3.4} + +2\,S_{1.2}Z_{3.4}$). Such families (Nos. 32—34) are summarized in Table 1 *d*.

Family 35 is of interest as a complement and correction. It was concluded in a previous paper (LUNDQVIST, 1956, Table 7) that family No. 1 was derived from the cross combination ho het × het het (mating category E.). The proof was based on its offspring family, No. 2, which was in turn obtained from the compatible sib intercross 1: 8 × 1 and segregated into three groups. Plant 1: 8 being female compatible must be judged a ho het plant. Inadvertently, plant 1: 1 was classified as ho het, although not definitely distinguished from the het het group, which will also give three groups in the offspring with ho het plant 1: 8. Thus, family No. 2 does not provide the basis for the same argument as given above by families Nos. 32—34 in Table 1 *d*.

Family 35 contains offspring derived from the two het het groups of family No. 1, crossed to het het sibs. Only three groups being obtained in family 35, it is definitely demonstrated that the ho het groups in family 1 were ho_ahet and ho_bhet, respectively, and that, accordingly, family 1 must be derived from the cross combination ho het × het het (*e. g.*, $S_{1.1}Z_{3.4}$ and $S_{3.2}Z_{3.4} \times S_{1.2}Z_{3.4}$ will in both combinations give $S_{1.2}Z_{3.3} + S_{1.2}Z_{4.4} + 2\,S_{1.2}Z_{3.4}$).

TABLE 2 a. *Segregation into het het and ho het plants in relation to number of groups distinguished and the expected ratio het het/ho het.*

Clone No.	Family Nos.	No. groups	Expected ratio het het/ho het	No. plants		Deviation	
				het het	ho het	χ^2	P
3	1, 2, 35	3	1 : 1	23	17	0.900	0.5—0.3
16	4, 5, 6	2	1 : 1	18	11	1.690	0,2—0.1
	7, 9	3	1 : 1	8	10	0.222	0.7—0.5
21	11, 12, 26, 27, 29, 30, 41	2	1 : 1	44	42	0.046	0.9—0.8
	16, 17, 18, 19, 20, 32, 33, 34	3	1 : 1	48	52	0.160	0.7—0.5
	15, 28, 38, 39	3	1 : 2	20	33	0.461	0.5—0.3

b. *Pooled data.*

Clone No.	Nos. of plants				Deviation	
	observed		expected		χ^2	P
	het het	ho het	het het	ho het		
3	23	17	20.00	20.00	0.900	0.5—0.3
16	26	21	23.50	23.50	0.532	0.5—0.3
21	112	127	110.67	128.33	0.030	0.9—0.8
Total	161	165	154.17	171.83	0.574	0.5—0.3
Sum of χ^2					1.462	
Heterogeneity, d. f. = 2					0.888	0.7—0.5

III. VIABILITY IN RELATION TO HOMOZYGOSITY FOR INCOMPATIBILITY FACTORS

For 27 of the 28 families considered, the genetics of incompatibilities was sufficiently cleared up to permit a prediction of the segregation patterns. Only family 8 of clone 16 is not sufficiently known to be considered here.

In Table 2 are given the segregations into the het het and the ho het categories. Of the three parental clones, No. 21 is the predominantly represented one. As may be seen from Table 1, three segregation patterns are theoretically possible within the families summarized in Table 2, *viz.*: (1) the het het and one ho het genotype, equally represented, (2) the het het and two ho het genotypes, all three equally represented, the ratio het het/ho het being 1/2, and (3) the het het and

two ho het genotypes, the het het genotype being twice as frequently represented and the ratio het het/ho het being 1/1.

It may be seen from Table 2 *a* that, irrespective of the segregation category, the ratio het het/ho het conforms quite well to expectation, as do also the pooled data within the three clones and the grand total material given in Table 2 *b*. A reduced viability of the homozygous category cannot be statistically demonstrated, but the slight deficit of ho het plants, obtained in all three clones, may warrant a certain attention.

IV. VEGETATIVE DEVELOPMENT IN RELATION TO HOMOZYGOSITY FOR INCOMPATIBILITY FACTORS

In all 28 families vegetative development has been studied in three characters: plant height, straw diameter, and tillering. By plant height is meant the longest straw measured in cm. from its base to the top of the ear; the straw diameter has been measured in tenths of mm. on the third internode of the straw with the biggest ear; by tillering is meant the number of well matured ears.

In 1954, 1955, and 1957 the plants were grown in beds and transplanted to pots in the greenhouse immediately before flowering. The families from 1956 were not grown under uniform conditions. Most of them wintered out of doors and were grown in boxes for the whole time. Families Nos. 26 (save four plants, two of them het het and two ho het, which will not be considered in this connection), 32, and 33, were grown in beds during part of the spring before being retransplanted to pots immediately before flowering. Nos. 34 and 35 were kept in boxes in the greenhouse for the whole time. All families were kept in the greenhouse at the time of flowering and afterwards.

Space does not permit all original data within the individual families to be presented, but the means for the het het and ho het groups of the individual families are summarized in Table 3. There are no clear and consistent differences between the groups; in fact, the higher mean values are about equally distributed between them.

An analysis of variance has been carried out (Table 4), the materials within the three clones being analysed separately. The families being of unequal size, the material was submitted to a hierarchical subdivision. Owing to the unequal treatment that the families received in different years, and considering the varying degrees to which the families have been inbred, differences between years and between families are to be

TABLE 3. *Means in three vegetative characters in relation to degree of heterozygosity for incompatibility factors.*

Clone No.	Year	Family No.	Number of plants		Means of					
					plant height in cm.		straw diameter in mm.		number of ears	
			het het	ho het	het het	ho het	het het	ho het	het het	ho het
3	1954	1	9	4	100.8	100.0	4.32	4.80	5.1	7.5
	1955	2	3	6	84.2	90.8	4.77	4.67	3.7	6.2
	1956	35	11	7	97.5	103.2	4.08	4.36	6.1	6.7
16	1954	4	7	3	94.6	99.2	5.24	5.37	5.7	4.7
		5	7	6	93.2	81.7	5.10	4.87	4.1	3.7
		7	6	3	93.3	99.2	4.57	5.23	5.0	6.3
		8	8	6	91.3	89.2	4.85	5.03	5.3	3.8
	1955	6	4	2	90.0	87.5	5.45	5.20	8.3	5.5
		9	2	7	90.0	75.4	5.02	4.85	4.5	2.9
21	1954	17	4	7	90.0	94.6	4.80	4.87	4.8	4.6
		18	6	8	101.5	94.4	5.30	5.35	7.2	6.3
	1955	11	4	6	82.5	85.0	4.80	5.23	3.5	5.7
		12	3	9	89.2	84.7	5.43	5.50	6.0	6.0
		15	2	4	87.5	72.5	5.00	4.35	6.0	5.5
		16	4	7	71.3	77.5	5.05	5.13	10.3	7.7
		19	2	5	75.0	69.5	4.30	4.90	4.0	3.8
		20	6	6	85.0	89.2	4.97	5.40	7.0	7.0
	1956	26	13	5	77.1	74.5	4.78	4.82	3.7	3.4
		27	6	4	123.3	115.0	4.87	5.25	3.5	3.5
		28	6	5	108.3	109.5	4.90	5.18	3.5	4.0
		29	5	4	99.5	98.8	4.90	5.45	3.2	3.5
		30	3	5	99.2	96.5	4.57	4.82	4.3	3.0
		32	11	8	76.1	76.3	4.08	3.93	3.7	2.9
		33	10	7	73.5	70.4	4.16	3.96	3.7	3.3
		34	5	4	106.5	103.8	4.46	4.60	3.8	6.3
	1957	38	6	6	91.7	91.7	5.97	5.83	5.5	3.8
		39	6	18	108.3	107.2	5.93	5.80	6.8	7.3
		41	8	7	117.5	116.1	6.00	5.93	10.8	9.6

expected *a priori*. Thus, it is evident that, in order to judge the differences between the het het and the ho het groups, the comparison between these groups must be made within families grown in the same year. The differences between the het het and the ho het groups do not reach

TABLE 4. *Differences in vegetative development between the het het and ho het groups. Analysis of variance in the material summarized in Table 3.*

Character	Clone No.	Source of variation	d. f.	Sum of squares	Mean square	Variance ratio	P
Plant height in cm.	3	c. Between het het and ho het	3	230.50	76.75	d/c 1.225	> 0.2
		d. Within » »	34	3196.50	94.00		
		Total	39	4350.00			
	16	e. Between het het and ho het	6	898.75	149.75	e/f 1.457	0.2
		f. Within » »	49	5040.25	102.75		
		Total	60	7773.00			
	21	e. Between het het and ho het	19	1165.50	61.25	f/e 1.445	0.2
		f. Within » »	197	17447.00	88.50		
		Total	234	72047.50			
Straw diameter in mm.	3	c. Between het het and ho het	3	0.9760	0.3252	c/d 1.056	> 0.2
		d. Within » »	34	10.4692	0.3080		
		Total	39	13.1188			
	16	e. Between het het and ho het	6	1.3596	0.2268	f/e 2.120	0.2—0.05
		f. Within » »	49	23.5584	0.4808		
		Total	60	26.9732			
	21	e. Between het het and ho het	19	3.9836	0.2096	f/e 1.641	0.2—0.05
		f. Within » »	197	67.7476	0.3440		
		Total	234	155.0008			
Number of ears	3	c. Between het het and ho het	3	29.96	9.99	c/d 1.232	> 0.2
		d. Within » »	34	275.73	8.11		
		Total	39	311.90			
	16	e. Between het het and ho het	6	27.74	4.62	e/f 1.301	> 0.2
		f. Within » »	49	173.90	3.55		
		Total	60	280.23			
	21	e. Between het het and ho het	19	67.60	3.56	f/e 1.357	> 0.2
		f. Within » »	197	952.04	4.83		
		Total	234	1980.11			

statistical significance for any of the three investigated characters. No correlation between vegetative development and homozygosity for incompatibility factors seems to exist in the present material of diploid rye, plants of the het het group and the pooled ho het category being equally well developed.

V. DISCUSSION

Neither in viability nor in vegetative development does homozygosity for incompatibility factors seem to be of disadvantage in the rye material studied. The material is small, to be sure, at the most only six different alleles together being represented at the S and the Z loci, respectively, in the descendants of the three investigated clones, and the total number of plants being only about 50 in two of the clones. No attempt has been made to study the four ho het genotypes, obtainable within a clone, in relation to each other. As may be seen from Table 1, the absolute genotypic assignment between the families will be too fragmentary, and the families themselves are generally too small to carry out such a study.

Only for clone 21 were mating combinations available, where competition between pollen grains with one and with no incompatibility factor common to the style could be studied. Four such combinations of various parentage were studied (families Nos. 12 and 26; 15 and 28; 38; 39), and in all of them, judging from the offspring segregation, the two pollen categories were equally efficient in producing fertilization. Although the exact genotypic relationships between the four combinations are not known, data indicate that, most probably, all four incompatibility factors of clone 21 may occur naturally in the homozygous stage. Thus it is not unexpected that, in this clone, homozygosity for the chromosome regions carrying these incompatibility factors had no especially deleterious effect.

It seems quite probable that the way of action of the incompatibility factors of clone 21 is representative of the population studied, and the evidence is strengthened by the two other clones, Nos. 3 and 16, showing no deleterious effect of homozygosity for incompatibility factors.

The accumulation of deleterious recessive genes that may occur, whenever production of homozygosity is prevented, is a well-known phenomenon. Most self-incompatibility systems previously described for Angiosperms, imply a more or less rigorous prevention of homozygosity for incompatibility factors, the most rigorous mechanism being

obtained for species where one locus has been postulated with autonomous (gametophytic) determination of the pollen specificities. Homozygotes for some factors, but not for all, will be formed naturally in other species, where the pollen specificities are determined by the anther tissues of the pollen producing plant (sporophytically); here dominance relationships between the alleles of the postulated locus, under appropriate conditions, will result in functioning and fertilization by pollen carrying a matched recessive incompatibility allele.

Data bearing on homozygosity for incompatibility factors in relation to viability and vegetative development are rather scarce in the literature. More or less clear cases of artificially produced homozygotes for incompatibility factors, displaying abnormalities, reduced vitality or a deficit in relation to the heterozygous siblings, have, however, been observed in species where the action of one gametophytic locus has been postulated. Such cases were found, *e. g.*, in *Nicotiana* (EAST and MANGELSDORF, 1926; EAST, 1927, 1934), and in *Petunia* (HARLAND and ATTECK, 1933; STOUT, 1952), *Trifolium repens* (ATWOOD, 1945), *Oenothera organensis* (LEWIS, 1949). In these species homozygotes for incompatibility factors do not occur naturally. In the distylic *Primula sinensis* the artificially produced dominant thrum displays reduced viability both in relation to the heterozygous thrum and the homozygous recessive pin (MATHER and DE WINTON, 1941). The pollen specificities are determined sporophytically, and the thrum homozygote is not produced naturally. No data could be found for species with one multiallelic »sporophytic» locus, but it might be expected that, where the dominance relationships are extended, the homozygotes mostly will not be at an essential disadvantage.

Although it may be difficult to exclude the possibility of a specific effect exerted by the incompatibility factor itself, this effect of homozygosity may be more readily explained from recessive detrimentals linked to the incompatibility locus. In view of the complicated structure of the incompatibility locus made probable for *Oenothera* and *Prunus* (LEWIS, 1949), and later on for other self-incompatible Angiosperm species, the suggestion that the incompatibility alleles might be various forms of structural defects (MÜNTZING, 1938) has lost much of its attractiveness; and EAST (1933) advanced genetical evidence against a similar suggestion. In any case it cannot apply to a breeding system as that in rye, where homozygotes for these factors may obtain naturally. And besides, not only may recessive deleterious genes accumulate in regions adjacent to the incompatibility locus, but there is also a

stronger selection for heterozygous advantage of linked favourable alleles close to the S locus (WILLIAMS and BROWN, 1956).

The occurrence of a similar effect of homozygosity, when the incompatibility system is of the rye type with two gametophytic loci, for which homozygosity may obtain naturally, could thus be of importance in the discussion of origin and evolution of such a system. In such a case it could be conceived that the two-loci system originated in some way through the union of two separate personate systems, each with one single locus, and that a certain accumulation of recessive deleterious genes in chromosome regions adjacent to these loci remained as a relict since that time. For, in its present way of action, there seems to be no reason for the system to promote an especial accumulation of such detrimentals in these regions.

Evidence of that kind was not obtained, however, for the rye material. Certainly, the fact that the ho het rye material matched the het het plants quite well does not eliminate the possibility that the prime origin of the two-loci system in rye may, in fact, have arisen by means of a more or less pronounced duplication. It is conveivable that the system might have been established for a sufficiently long time to permit a complete levelling of a previous accumulation of deleterious recessive genes close to the incompatibility loci. The occurrence of similar two-loci systems in the 14 chromosome diploid grass species *Festuca pratensis* (LUNDQVIST, 1955 and unpublished) and *Phalaris coerulescens* (HAYMAN, 1956) also speaks against these systems having a recent origin.

It should be observed here that the above considerations hold true only in the case of more than one allele being represented at both loci in the interbreeding unit. As soon as only one allele is left and homozygosity prevails at one locus, the other locus will exert the effective control of incompatibility, and homozygosity will be prevented here. Thus deleterious recessive genes may now accumulate in regions adjacent to the permanently heterozygous locus.

The interpretation of incompatibilities on the personate scheme implied one incompatibility locus with gametophytic determination of the pollen specificities, but even for the classical material in *Nicotiana*, presented by EAST and MANGELSDORF (1925), it is not quite clear whether only one incompatibility locus may be at work (EAST, 1926, 1929). The investigated material originated from a cross between *Nicotiana Forgetiana* and *N. alata*. A very high frequency of cross-compatibility occurred in the F_2 and following filial generations (EAST

and PARK, 1917), and only after continued inbreeding could a genetic analysis be performed, the results conforming to the scheme of one multiallelic locus controlling incompatibilities gametophytically.

In view of the advantage over the one-locus system inherent in the two-loci system acting on the rye scheme (LUNDQVIST, 1954, 1956), it might be conceivable that in fact two such loci controlled the incompatibilities in the investigated *Nicotiana* material, one of them being rendered homozygous through intensive inbreeding. What speaks against this interpretation is the deficit of homozygotes, common in the offspring from selfed incompatibility heterozygotes in the material in question (EAST and MANGELSDORF, 1926; EAST, 1927, 1934). And it is improbable that two separate single loci, introduced by each of the two crossed self-incompatible species, could establish an immediate and successful co-operation on the rye scheme in the F_1 hybrid. What caused the complications in the first hybrid generations might justify a reinvestigation. It should be observed, however, that many complications that are not immediately relevant to the action of the oppositional factor system may obtain in hybrids of that kind (*cf.* AR'RUSHDI, 1956).

It is suggested that better knowledge of the effect of homozygosity for incompatibility factors may give valuable information about the mechanism of the incompatibility control in various materials.

SUMMARY

From three clones of diploid Steel-rye, all previously proved to be heterozygous at the two incompatibility loci operating in rye, 28 families, altogether, were obtained during 1954—1957, within which plants heterozygous at both incompatibility loci (het het) could be compared with siblings belonging to the ho het category. The plants were compared in viability and vegetative development (plant height, straw diameter, and tillering), and, in all, about 350 plants were studied.

Neither in viability nor in vegetative development does homozygosity for incompatibility factors seem to be of disadvantage in the rye material studied. In view of the observation, specifically made within one of the three clones, that a single incompatibility factor common to pollen and style will cause no functional disadvantage to the pollen carrying it, it is concluded that homozygosity for incompatibility factors may obtain naturally in the population and that, accordingly, there is no especial opportunity for deleterious recessive genes to ac-

cumulate in regions adjacent to these loci. Thus, it is not unexpected that homozygosity for the chromosome regions carrying these incompatibility factors had no especially deleterious effect such as might be displayed in species where homozygotes for incompatibility factors do not occur naturally.

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BRIEF REPORT

B. BJURMAN: Note on the frequency of univalents in some strains of *Triticale* and their hybrids.

(Received September 14th, 1957)

The material studied consists of two strains of rye-wheat ($2n=56$), *Triticale* A and C, their F_1 hybrids and a uniform recombination product, derived from a cross between these strains. *Triticale* A is identical with *Triticale* Rimpau, *Triticale* C is derived from Russia (*cf.* MÜNTZING, 1939). The constant recombination product was raised by professor MÜNTZING, the primary F_1 hybrids by myself. The frequency of univalents at first metaphase of meiosis in *Triticale* A and C was previously studied by MÜNTZING (1939, p. 390) who found meiosis in *Triticale* A to be much more irregular than in *Triticale* C. The average frequency of univalents in the former strain was found by him to be 6.10, the corresponding value of *Triticale* C being 1.88. Hybrids between these two strains were observed to be more irregular than *both* parents, the average frequency of univalents being 8.57 (MÜNTZING, 1939, pp. 404—405).

As this observation seemed to require further studies on a larger material, the present investigation was undertaken. The frequencies of univalents at first metaphase of the p.m.c. were determined on permanent squashes as well as sectioned material. The data accumulated are given in Tables 1 and 2.

From the tables it is evident that in each year of observation the frequency of univalents is, indeed, higher in *Triticale* A than in *Triticale* C, this difference being significant ($t=5.47$). The data in Table 2 also verify the most interesting point, viz. that the hybrids between the two strains are much more irregular than either parent. Thus, the average frequencies in A×C and reciprocally were found to be 4.28 and 4.08 respectively, the corresponding values of the parents being as low as 1.47 (*Triticale* A) and 1.05 (*Triticale* C). These latter values are from plants derived from intra-strain crosses, A×A and C×C, these plants being in all respects identical with ordinary members of the respective strains.

In general the values obtained by me show lower degrees of meiotic irregularity than those reported by MÜNTZING (*l. c.*). The reason for this is unknown. It should be observed, however, that the frequency of univalents is very labile, strong differences being observed between plants and even between different spikes of the same plant (*cf.* also MÜNTZING, 1957). This is indicated in the tables by χ^2 values representing various degrees of significance. Also differences between years were apparent, but did not reach the level of significance.

In the constant recombination product, derived from the cross between the two *Triticale*-types under consideration, meiosis was found to be less irregular than in the primary hybrids, the average values obtained being of the same magnitude as in *Triticale* A. Finally it should be mentioned that one of the univalents represents a special, recognizable type with a median constriction. A similar chromosome was observed by LAMM (1936) in inbred rye. This is in

TABLE 1. *Frequency of univalents in Triticale A and C and in a recombined derivative of these strains.*

Material	Year	Number of univalents at first metaphase of meiosis															n	M
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Triticale A Plant 1	1954	37	8	16	8	10	3	1	1	—	2							86	1.74
C×A -der. Plant 1	»	29	34	72	25	25	6	5	1	1	—	1	—	1				200	2.25
» 2	»	35	7	16	8	5	2	1										74	1.34
		64	41	88	33	30	8	6	1	1	—	1	—	1				274	2.00
										$\chi^2=32.66^{***}$								
Triticale C Plant 1	»	120	9	18	—	2	1	1	1									152	0.47
» 2	»	122	28	58	13	28	4	8	1	3	2	2						269	1.52
		242	37	76	13	30	5	9	2	3	2	2						421	1.05
										$\chi^2=50.12^{***}$								
Triticale A Plant 1 Spike 1	1955	53	12	33	5	27	3	4	1	5	—	4	2	1	—	2	1	153	2.64
« 1 « 2	»	106	89	94	23	33	3	9	—	3	9							369	1.77
		159	101	127	28	60	6	13	1	8	9	4	2	1	—	2	1	522	2.02
										$\chi^2=22.06^{***}$								
C×A -der. Plant 1	»	28	9	15	3	2	1	1										59	1.14
» 2		5	5	8	6	5	3	1	1	1								35	2.71
		33	14	23	9	7	4	2	1	1								94	1.72
										$\chi^2=18.55^{***}$								
Triticale C Plant 1 Spike 1	»	188	22	59	12	16	1	—	1	1	3	1						304	0.98
» 1 » 2		158	21	54	12	12	1	4	—	—	—	1						263	0.96
		346	43	113	24	28	2	4	1	1	3	2						567	0.97
										$\chi^2=0.24$								

TABLE 2. *Frequency of univalents in Triticale A and C and in their F_1 hybrids.*
(All observations made on material fixed in 1956)

Material	Number of univalents at first metaphase of meiosis															n	M
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Triticale A Plant 1	33	20	42	7	11	1											114	1.53
(A×A cross) » 2	40	8	33	5	4	1	1										92	1.26
» 3	47	22	30	9	17	1	2	1									129	1.57
	120	50	105	21	32	3	3	1									335	1.47
									$\chi^2=9.66$									
Triticale A × C Plant 1	3	3	24	16	28	17	15	3	3	1	1						114	3.95
» 2	1	4	15	14	30	7	20	5	5	4	1						106	4.45
» 3	—	8	19	10	27	8	15	4	9	1	5						106	4.46
	4	15	58	40	85	32	50	12	17	6	7						326	4.28
									$\chi^2=2.53$									
Triticale C × A Plant 1 Spike 1	—	1	6	21	35	12	18	7	4	2	2						108	4.66
» 1 » 2	—	1	8	14	30	27	23	4	4	2	1	1					115	4.79
» 1 » 3	8	14	14	21	20	14	8	6	—	2	—	—	—	1			108	3.48
	8	16	28	56	85	53	49	17	8	6	3	1	—	1			331	4.08
									$\chi^2=46.89^{***}$									
Triticale C Plant 1 Spike 1	52	16	20	5	3	3											99	0.99
(C×C cross) » 1 » 2	60	8	30	5	11	2	2	—	1								119	1.32
» 1 » 3	60	22	27	5	4												118	0.91
	172	46	77	15	18	5	2	—	1								336	1.05
									$\chi^2=6.55$									

accordance with the fact that the unpaired chromosomes in *Triticale* are, at least predominantly, rye chromosomes (MÜNTZING, 1957).

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